PHYSIOLOGICAL INVESTIGATIONS INTO THE ACTION OF DDT

2,2-BIS(P-CHLOROPHENYL)-1,1,1-TRICHLOROETHANE

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE WIS- EN NATUURKUNDE AAN DE RIJKS-UNIVERSITEIT TE UTRECHT, OP GEZAG VAN DE RECTOR MAGNIFICUS DR. H. WAGENVOORT, HOOGLERAAR IN DE FACULTEIT DER LETTEREN EN WIJSBEGEERTE, VOLGENS BESLUIT VAN DE SENAAT DER UNIVERSITEIT IN HET OPENBAAR TE VERDEDIGEN OP MAANDAG 30 MEI 1949, DES NAMIDDAGS TE 4 UUR

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VOORWOORD

Een ieder heeft reeds in zijn jeugd kennis gemaakt met de grote voldoening, die een goed geslaagde analyse hem verschaft: oh, wat hebben wij een massa speelgoed gesloopt.

Toch beseften wij al vroeg, dat de brokken, die ééns onze pop of locomotief waren geweest, iets onbevredigends hadden: wij bemerkten echter tot onze schrik, dat de synthese slechts zelden gelukte.

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INTRODUCTION

The search for new and more effective chemical insecticides is generally undertaken rather unsystematically, owing partly to a lack of insight in the nature of toxic action, partly to other factors. Our knowledge of the relation between chemical structure and toxic properties is incomplete, in a number of cases even the existence of such a relation is doubtful. Neither do we know very much about the relations between toxicity and physical properties.

The limitation of our insight is further due to the fact that we know very little about the normal physiology of insects. Yet such knowledge is a prerequisite to understanding toxic action.

The facts just mentioned keep many people from basing their method of investigation on existing physiological and toxicological views. Especially in America, but also in other countries, entomologists are looking for new insecticides by a mere process of hit or miss elimination. A certain chemical factory produces a series of by-products, which, before being considered as waste-matter, are, among other things, tested for insecticidal activity. Out of hundreds of products thus examined one or two may be left that are in some degree satisfactory. Often some modifications are then brought about in the molecule, but as a result of the above-mentioned lack of insight in the relation between structure and toxicity, this is mostly done without following a clear line of thought. If in this way a good insecticide is found, it is really more a question of hit than of wit.

Although the difficulties attending a systematic research founded on our knowledge of physiology and toxicology are thus avoided, it will be clear that the systematic method should be preferred in future, despite the limitation of its foundation.

The future begins today, and it is of importance, therefore, to undertake an investigation on these lines forthwith. In doing so it will appear that, in contradistinction to architecture, the building, as it grows, stimulates its foundation to further development.

It is one of the great merits of the "Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek" [1]), that it stimulates such

[1]) National Council for Applied Scientific Research.

fundamental research and even gives it material support. Not until the importance of such research is appreciated in wider circles, can palpable results be expected.

The present investigation was part of a more comprehensive enquiry into the physiological action of insecticides, which is being carried out under the auspices of the "Organisatie voor T.N.O.". In this connexion we have paid more attention than was perhaps strictly necessary to the literature on the subject, for we intend this publication to be also a review of the relevant articles in continuation of the two previously published "Overzichten over de literatuur betreffende DDT" [1]). If necessary a supplementary report will be published in due course.

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[1]) Published by the "Landbouw-Organisatie voor T.N.O.", 12 Koningskade, The Hague (1947/'48).

THE PROBLEM

The search for new insecticides following the above-mentioned principle may be undertaken in two different directions.

It is possible, following a comprehensive study of the physiology of insects, to seek an opportunity to intervene in the normal course of events. But it is probably more fruitful to link our investigation with insecticides that are already known. Our effort will then be directed to answering these two questions:

1. In what way does a given insecticide act?
2. Can we, on the strength of our insight into that action, effect improvements?

Now the answer to the first question may be so difficult, that for the time being we cannot consider solving the second one. In the light of our earlier remarks this is not surprising, nor shall it prevent us from pursuing our investigation in the direction we have chosen.

The present investigation deals with the first question, and the insecticide under consideration is 2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane, generally known as "DDT".

As a rule it is preferable to call this compound p,p′DDT and to use the abbreviation "DDT" only for the crude product, as the latter also contains non-active isomeres, e.g. o,o′DDT, o,p′DDT, m,p′DDT etc. [56][1]). As we shall be dealing almost exclusively with p,p′DDT in the following pages, we shall nevertheless, for convenience' sake, indicate this compound by DDT. When a different isomere is intended, this will be pointed out.

A further analysis of our problem teaches us that we have to formulate it in a few other questions. When we try to understand what, properly speaking, is meant by "the action of an insecticide", this appears to be no simple question.

DDT, applied as a contact poison, acts almost exclusively on Arthropodes. This specificity may be considered as the most characteristic feature of its action.

But after injecting it into the body, no question of specificity arises; in all sorts of animals examined, similar symptoms occur and the lethal dose lies within a reasonably small range. Also the

[1]) Numbers between brackets refer to the bibliography on page 109.

creation of DDT symptoms may therefore be considered as the most characteristic feature of the action of DDT.

Finally, the cause of death after DDT poisoning may be taken as a criterion for its action. That in case of poisoning the cause of the symptoms need not necessarily be the cause of death, will be generally known (for example the violent spasms after strychnine poisoning in mammals are due to an abnormally strong reflex-activity of the motor centres; but since also the respiratory muscles are in a spasm, the cause of death is asphyxia).

Thus we now formulate our problem as follows:

1. What is the cause of the specific action of DDT as a contact poison in insects?

2. How are DDT symptoms produced?

3. What is the cause of death in case of DDT poisoning?

This analysis of the statement of our problem is not yet complete. For it is not certain, in some cases even improbable, that a poison, in the case of acute intoxication, gives the same symptoms and the same cause of death as in chronic poisoning. We confine our investigations to *acute* poisoning and we wish it to be clearly understood that our results and conclusions concern only this kind of poisoning. How far existing views on the consequences of chronic poisoning may be affected by our investigations is beyond the scope of this study.

Sufficient attention has been paid to the statement of our problem, in so far as it is connected with our own work. In the course of this study we shall also have occasion to refer to some fields of literature, which, although bearing upon physiological problems connected with DDT, fall outside the compass of our own investigation. It is impossible to refrain from casting an occasional glance into these neighbouring fields, but we hope that the text will show clearly what was essential to us.

Chapter I

THE SPECIFIC ACTION OF DDT AS A CONTACT POISON

The contact poisons we are dealing with penetrate into the organism through the skin and then excercise their toxic action.

Generally speaking, a toxic action will only occur when the rate of penetration is higher than that at which the quantities absorbed are excreted or detoxicated. From which it appears that penetration on the one hand and intermediary metabolism on the other are mechanisms which should retain our full attention. We shall return to the latter mechanism in Chapter II.

As an explanation of the specific action of DDT as a contact poison in Arthropodes we can imagine two extreme possibilities:

1. A relatively *low concentration* of DDT in the internal environment of Arthropodes already produces a *toxic action*, while the process of penetration does not essentially differ from that in other animals.
2. The toxic concentration in the internal environment is about the same in all kinds of animals, but *penetration* in the case of Arthropodes is a quicker process.

It is true that combinations of the two extremes are conceivable, but in this way the problem is stated as sharply as possible. The answer to our question is to be obtained by examining the toxicity of the agent after injection in insects and in other animals. For in this way we best determine its "real toxicity", without the interference of the mechanism of penetration.

It is beyond the scope of our argument to go further into the concept of "toxicity", although it would by no means be superfluous to state its meaning more precisely. We hope to return to this problem in a later publication, but for the present let us adopt as a measure of toxicity (and at the same time as a measure of the susceptibility of the animal) the median lethal dose (MLD or LD50), this being the dose at which 50 per cent of the test animals die. It goes without saying that the LD50 of a certain poison differs with different species, while it is also to a high degree dependent on the circumstances under which the experiment is carried out. Therefore, in comparing an LD50 for different species it is of importance that the

conditions in all experiments are the same, as far as possible. Nevertheless there will always be factors beyond our control, which lessen the comparability of the data for different species. Thus it is very likely, for example, that the water-content of an animal affects the LD50 calculated per gram animal, since the dilution of the poison injected differs in animals of different water-content. Yet it is not possible to ascertain the influence of this factor merely by taking the water-content into account. Only a certain part, but we do not know how much, will affect the concentration of the poison injected.

Similar remarks hold for the relative weight of the skeleton, and there are still other factors of the same order which may weaken the comparability of the LD's 50. There is, for example, the fat content of the animal; fat of course affects the animal's weight, but we can hardly consider this fat as belonging to the living substance of the animal and thus requiring a proportional quantity of poison. Besides, we know that in the case of DDT for example, there can be a considerable accumulation of the poison in fatty tissues, owing to which the comparability of results in animals having a different quantity of body fat becomes dubious.

Therefore, wanting to know exactly how much poison is required in the organs involved to produce the specific effect, is evidently asking too much.

Even if we should isolate the organs in question — supposing that we knew them — and continue the investigation on those lines, we should not come any further, for it is hard to see how from these data we could obtain a clear conception of the processes occurring in the intact animal. For example, in the case of perfusion experiments with isolated organs a constant concentration is maintained, whereas in the intact animal the concentration must necessarily vary during the process of poisoning.

From the foregoing it may appear that when comparing the LD50 for various species we may not entertain too high expectations as regards the measure of agreement between the data.

In other words, when a poison has exactly the same "real toxicity" for two different species, it is by no means certain that the LD50 should be the same in the two cases. For want of something better we shall therefore have to accept an approximate agreement of the LD's 50 as an indication of the toxicity being "approximately the same" for the two species.

Determination of the LD50 after injection

Material

Most of the experiments were undertaken with adults of *Periplaneta americana* and *Rana esculenta*. The cockroaches came from "Artis" [1]) where they have always been found as vermin in the warmer cages.

From the reproducibility of the results during three years we know that the material does not show any significant seasonal fluctuations. Both the distribution of the sexes in a population and the rate of reproduction of *Periplaneta* were found to be very regular the whole year round. Therefore we did not need to take into account any possible difference in susceptibility between males and females, or between individuals of different age.

To prevent any misunderstanding we must anticipate a moment what is mentioned under the heading "Results" (p. 26) and in Table II. From what has been said one might get the impression that it took us more than three years to determine the LD50 of DDT. This was of course not the case. In the course of the said three years, however, using the technique and method described, we carried out extensive determinations of the LD50 for all kinds of other insecticides, administering many thousands of injections.

Thus were determined the LD's 50 of different derris-root preparations, of pure rotenone, and of various synthetic insecticides, e.g. "Velsicol 1068", hexachlorocyclohexane, tetra-ethylpyrophosphate, "Thanite".

The results of these tests have partly been published [58], another part will be published later. The determination of the LD50 of DDT by means of injections, as far as technique and method are concerned, was thus only part of other, more comprehensive investigations.

It is of importance that the temperature of the animals on their way to the laboratory does not sink below about 15° C., as lower temperatures may occasion a high rate of mortality. In the laboratory, when kept in a ventilated thermostat at 30° C., they easily remain in good condition for 3 to 4 weeks. They were fed six times a week on a mash consisting of wheatmeal (1000 g.), whole milkpowder

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[1]) The Amsterdam Zoological Gardens.

(900 g.), dried yeast (100 g.), Vit. C (500 mg.), "Davitamon A" (10 ml.), mixed in water.

There was always a dish of water in the thermostat.

Technical provisions

Injection-liquid

DDT does not dissolve in water [1]), but only in lipids and fat-solvents. This makes our choice of the injection-liquid a little difficult.

Most fat-solvents are themselves toxic and fatty oils are too viscous to pass through the narrow hypodermic needle. Efforts to make stable suspensions of DDT in water (or a saline) have failed so far. So have a series of experiments in which it was tried to make a stable suspension by means of ultra-sonic waves produced by a piezo-electric quartz crystal generator [2]).

As a starting-point solutions of DDT in acetone of various concentrations (5 to 40 per cent) were used. Part of it was poured out into water; in one experimental series the test-tubes were exposed to ultra-sonic waves while the solution was being added, in another series they were exposed shortly after this had taken place. Also a reversed order, in which water was added to the acetone-solution (the other conditions being unchanged) was tried. The best that could be attained in this way, was still far from satisfactory. After a comparatively short time (one hour to one day) a varying quantity of DDT always coagulated.

It is possible that this method will yet yield good results if other frequencies and other powers are used. In our case the frequency was always 220 kc., while the ultra-sonic energy reached the test-tubes with about 10 watts. The experimental conditions, however, did not allow many variations.

It was therefore necessary to use emulsions. Most emulsifiers, however, have themselves a toxic action. At last we succeeded in composing an emulsion satisfying all requirements. We proceeded as follows: p,p'DDT was dissolved (the temperature being slightly raised) in oleum arachidis (peanut oil) up to a maximum concentration of 10 per cent. Part of this solution was emulsified with gum arabic and a saline.

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[1]) 0·00025% [55].

[2]) These experiments were carried out for us in the laboratory of the "Physisch-Technische Dienst" (Technical Physics Department T.N.O.-T.H.). We here acknowledge our sincere gratitude to Messrs. H. de Zeeuw and J. v. d. Harst.

For the injection of *Rana* common Ringer's solution was used, the saline for the cockroach being constituted as follows: 9·82 g. NaCl + 0·77 g. KCl + 0·50 g. $CaCl_2$ + 0·18 g. $NaHCO_3$ + 10 mg. NaH_2PO_4 + 1 g. glucose, dissolved in 1000 ml. distilled water. The *p*H was adjusted to 7·0 to 7·5 by adding a small amount of NaOH or HCl.

To every ml. of oil 1·5 to 2 g. of gum arabic should be used. Both by diluting this stock emulsion in varying degrees, and by varying the DDT-content in the peanut oil, the quantity of DDT per ml. of emulsion could be altered at will. But actually we nearly always used the following concentrations: 0·5‰ p,p′DDT, 0·5% oleum arachidis, 0·75% gum arabic. Such an emulsion can be held for 2 or 3 weeks.

In the control emulsions peanut oil without DDT was used.

Injections

Injecting frogs presents no difficulties. The injection-liquid was always brought into the dorsal or lateral lymph sac.

Injections of the control emulsion in quantities 1·5 times or twice as large as the largest quantity of DDT emulsion we ever injected in a frog, were completely harmless.

A handicap in injecting a great number of cockroaches in a short space of time is the great mobility of these animals. This may be counteracted by applying a narcosis (e.g. with CO_2, cyclopropane or ether [18, 115, 171, 173]) or extreme cold, but we did not prefer these methods on account of their possible harmful effects. In a specially designed apparatus (fig. 1) the animals could quickly be fixed and released, without any harm to the body.

A second difficulty is finding a convenient diameter and a suitable material for the needle.

Needles that are too thick make too large a perforation, whereas needles that are too thin soon get clogged with solid impurities of the gum arabic. Glass needles often break. Best results were obtained with an ordinary hypodermic needle no. 20 (0·5 mm outside ⌀) [1]).

The needle was always inserted a little laterally between the tergites of the abdomen, mostly between the 3rd and 4th, or the 4th and 5th.

The intention is to bring the liquid straight into the body cavity, so that the emulsion can there mix with the haemolymph. The

[1]) Made by "Dr. A. WANDER S.A.", Berne-Switzerland.

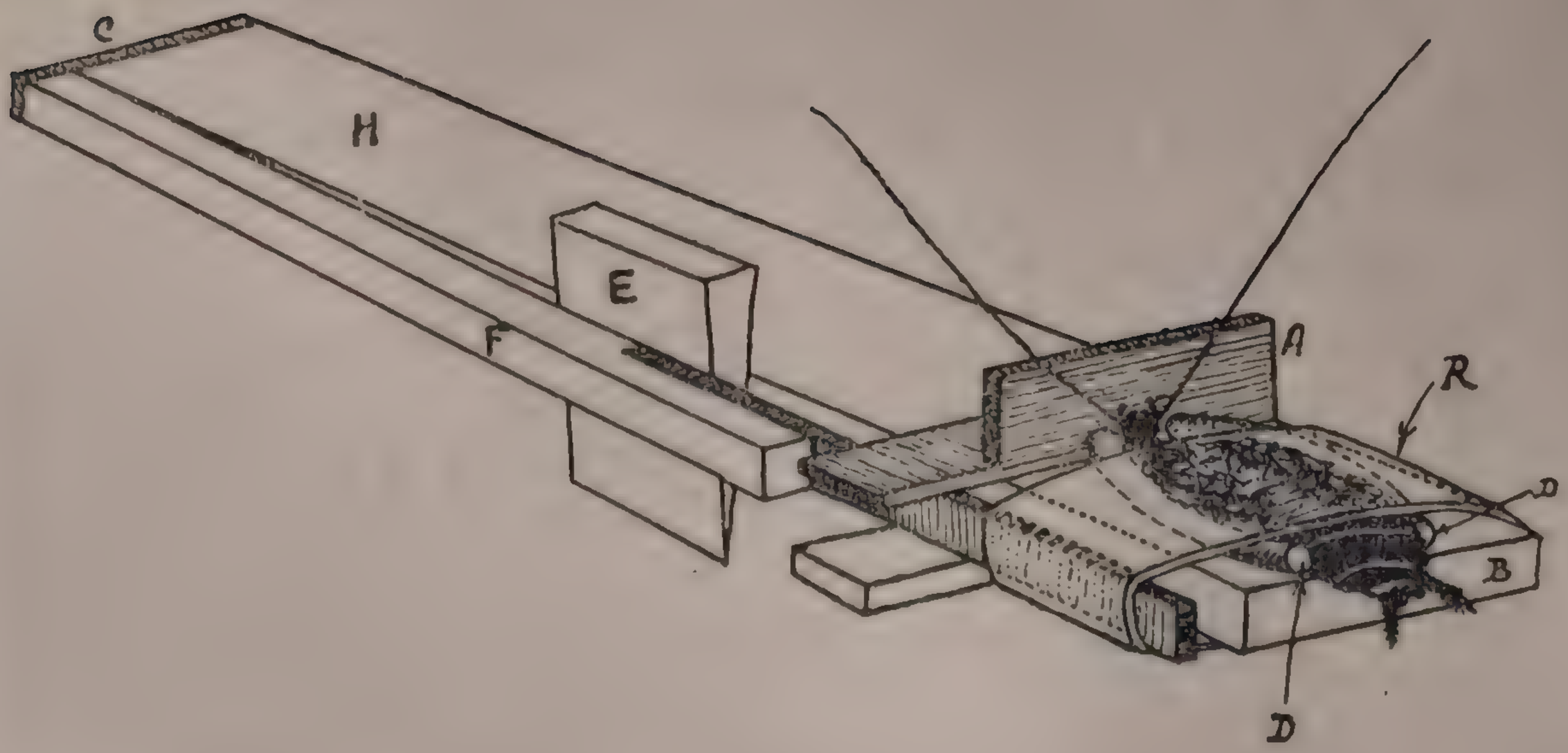


Fig. 1

The animals are laid upside down on a table *B*. They are fixed in place by lateral metal strips *D*, which are attached to a metal support *A*. By means of a wedge *E* between *F* and *H* the rubber band *R* can be tightened. (*F* is pivoted at *C*.)

question can be raised, however, whether, with the above procedure, this actually happens, or whether all or part of the liquid is injected into the intestine.

To answer this question a number of animals were injected with Indian ink. After dissection of such animals ink was hardly ever found in the intestine. It appeared further from these tests, that the liquid spreads very rapidly throughout the body; after 15 to 30 seconds we find the ink even in the distal segments of the tarsi.

The perforation caused by the injection is hardly mortal in itself, as can be seen from TABLE I. Neither is the additional quantity of liquid which the animal receives by the injection in itself an important factor. This appears from the results obtained after injection of as high a quantity as 120 mm^3 of insect-saline (TABLE I).

It should be borne in mind that a cockroach weighs about 1 g., so that 120 mm^3 would correspond to about 9 l. in a man of 75 kg.!

Some preliminary tests further showed that these animals can be inflated with more than 120 mm^3 of air without showing any harmful effects, and that they also bear tolerably well a dilution of their body fluids with about 100 mm^3 of distilled water. Such figures illustrate very clearly, how great a resistance our test animals have and how little we need fear a harmful influence of the injection as such.

TABLE I

Periplaneta. Influence of the injection as such.

No.	Injection	Volume in mm^3	Number of animals	Per cent mortality after 4 days
1	Untreated controls	—	Hundreds	<2
2	Single prick	—	20	5
3	Insect-saline	120	20	5
4	Air	120	20	5
5	Distilled water	100	38	16
6	Control emulsion	120	20	5

Finally the TABLE shows that the control emulsion does not produce any appreciable mortality either.

Now, whether the prick and the increase in volume are really harmless cannot be concluded from these data. The possibility remains, that, without being lethal factors, they yet produce a slackening of general resistance, which causes our LD_{50} to be too low. This, however, need not trouble us, as this influence is certainly constant. So our test animals may actually be a little less susceptible than we think.

Meanwhile it is very likely that the slight mortality in those animals (TABLE I) was solely caused by the perforation. For, apart from the injection with distilled water, mortality was never higher than that after one single prick. And in the individuals that died we mostly noticed a big rupture between the tergites, on the spot where they had been injected. It is possible that such animals suffered from a wound-infection that was responsible for their deaths.

Some animals lose a little liquid (haemolymph, injection-liquid or both) during the injection. This occurred far less frequently when the animals were used after a 24 hours' starvation. (They can very easily go without food for 1 to 2 weeks.) This is due to the fact that after such a period the animals were far more "relaxed" than before. If nevertheless failures did occur during the experiment, we did not include them in our calculations. This happened in 1 to 3 per cent of the cases.

As we used starvation only as a technical device, we deemed it unnecessary to go further into the causes of the effect produced.

Injection apparatus

We used normal syringes of 1, 2, and 3 ml. Verifying these syringes they were found to be highly cylindrical. This enabled us to construct an apparatus, based on the VAN DAM'S micro-pipette [44], by which very small quantities can be measured (fig. 2). In the syringe of 1 ml. for example, one revolution of the screw was equivalent to 26·7 mm³. With this apparatus the possible error need not exceed 0·5 mm³: in an injection of 26·7 mm³ this represents about 2 per cent.

Fig. 2

Weighing

To weigh a great number of cockroaches within a short space of time, we must dispose of a quick-acting apparatus. A simple torsion-balance with a wire of suitable dimensions fitted our purpose.

By it the weight of every animal could be determined with a margin of error of 1 per cent within the space of about 30 seconds.

METHODS

For every test more than 100 animals were used. They were marked (by punctures in the wings) and put in boxes (five animals in each) in the thermostat, without food. After 24 hours the animals were weighed and injected. Every day the mortality was noted down and on the 4th day an account was made up. This period of 4 days, from a theoretical standpoint, is entirely arbitrary. Experience tells us, however, that after that period a change in the rate of mortality is extremely rare. Other investigators, too, in similar research, have kept to this period [105].

When all animals receive the same dose *(d)*, say one complete revolution of the screw, the dose per gram animal *(x)* is different for each animal. The weight of an animal *(w)* is in each test proportional to the readings of the torsion-balance (arbitrary units).

Be s such a reading, than we can write:

$x = \frac{d}{w}$ and $w = a.s$ (a representing a constant), so $s.x = C$ (C is a constant, viz. $\frac{d}{a}$).

In other words, the relation between s and x plotted on a double logarithmic scale, is a straight line. The slope of this line can in each test be easily determined from the quantities d and a. The value of a is determined in each test from the calibration curve of the torsion-balance.

In fig. 3 it is demonstrated by an arbitrarily chosen example how by means of such a graph we divide the material into a number of *groups*; the mortality within each group gives a point for the dosage-mortality curve. When fixing the demarcations of our groups we must consider two counteracting principles: if the group is too small, the number of animals is insufficient for a reliable calculation of the mortality within that group; if, on the other hand, the groups are too large, one finds too few points for the dosage-mortality curve.

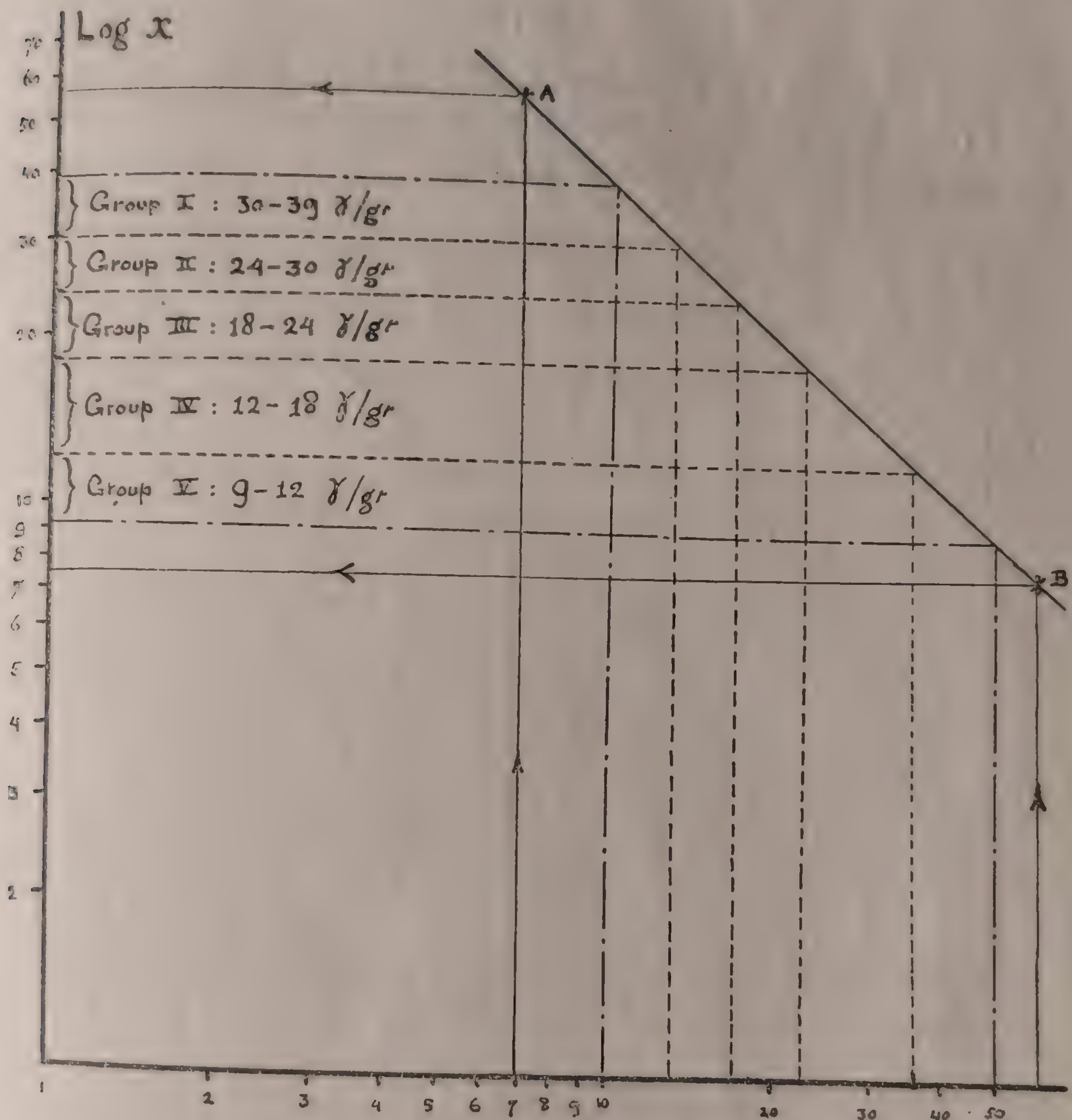


Fig. 3

The line AB is determined by the values for x corresponding to two arbitrary values for s, to be calculated from the expression: $x = \frac{d}{as}$, in which d = dose, a = constant (see text), s = reading of the torsion-balance.

The LD50 can, as is known, be calculated from the dosage-mortality curve by interpolation. This curve being asymmetrically S-shaped, many points are needed to fix it accurately. Thanks to the theoretical considerations of BLISS [16, 17] and others [64, 111] we know that of this curve the traject between 40 to 90 per cent kill can be transformed into a straight line. Our findings are therefore plotted on a logarithmic probability scale, instead of on a linear coordinate system. Thus we need far fewer points; as a rule 4 will be sufficient.

RESULTS

TABLE II shows the combined results of four determinations of the LD50. The figures of this table are graphically represented in fig. 4 on a logarithmic probability scale.

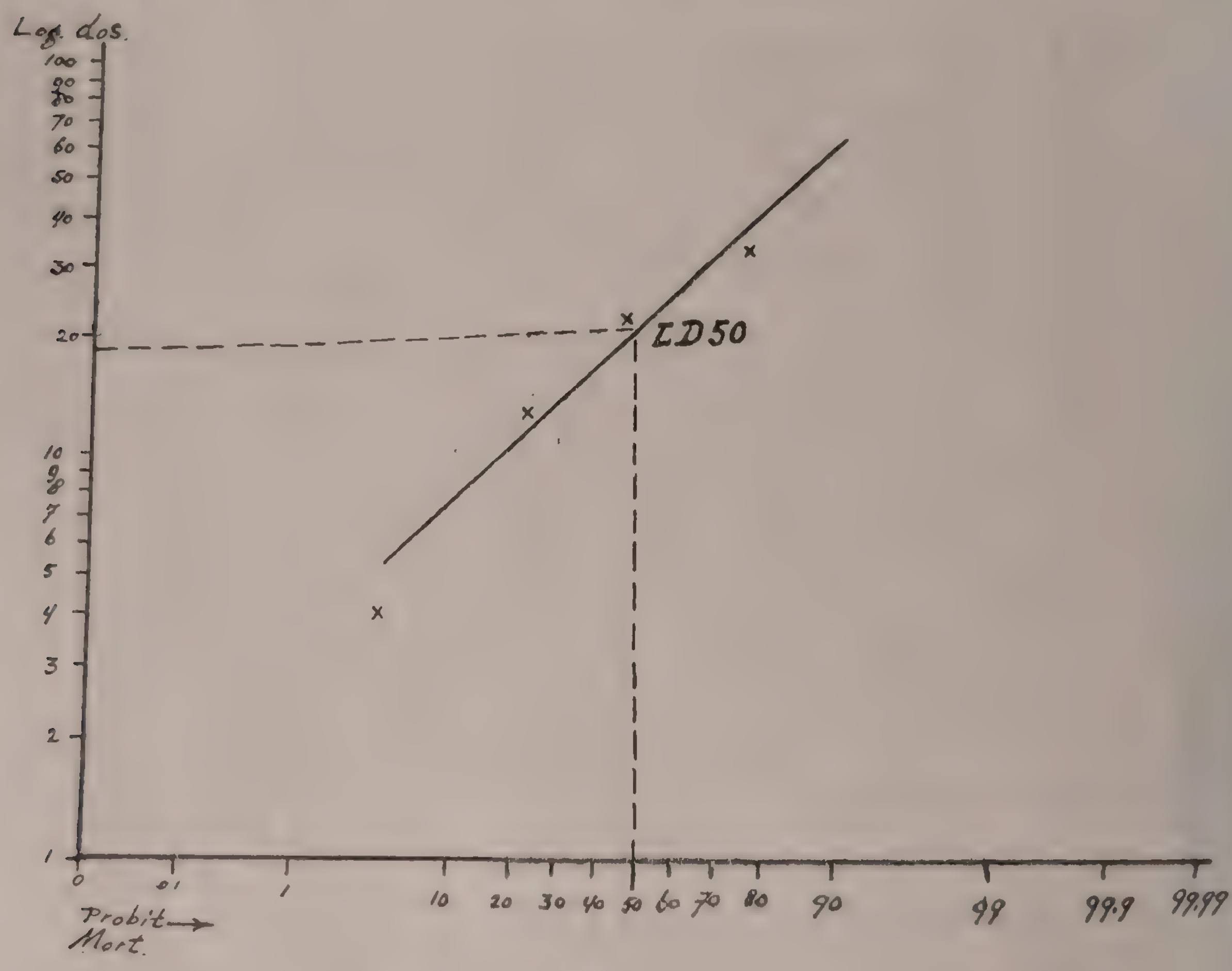


Fig. 4
For explanation see text.

We see from this that the LD50 of DDT is approximately 20 γ (micrograms) per g. b.w. (body weight). This value was invariably found in experiments where the LD50 of DDT for cockroaches had

to be determined, for example, when the toxicity of another compound had to be compared with that of DDT. We do not intend to discuss these investigations here, as they refer to quite a different problem.

Table II

Periplaneta: Mortality after injection of DDT.

γ/g. b.w.	Number of animals	Per cent mortality after 4 days [2]
0 [1]	80[1]	0
0—8	87	4·5
8—16	78	23·0
16—24	156	49·0
24—32	66	78·0
32—40	13	100

[1] These animals only received control emulsion. See p. 21.

[2] Mortality of the controls reduced to zero $\left(\frac{a-b}{a} \times 100\right.$, in which a is the percentage surviving in the controls, b that surviving in the actual test).

As an exact determination of the LD50 in *Rana* would also cost hundreds of animals, we did not pursue the matter. But from our findings it appears, that the LD50 here is certainly in the neighbourhood of 10 to 20 γ/g. b.w. and on no account higher than 20 γ/g. b.w. An extract from these data is shown in Table III.

Table III

Toxicity of DDT to *Rana*, after injection.

γ/g. b.w.	Number of animals	Results
0[1]	2	Still alive after one week
5	2	" " " " "
10	3	Two of them died in 24 hrs., the other one lived longer than one week.
15	2	Dead in 24 hrs.
20	1	" " 24 "
> 20	18	All died within 24 hrs.

[1] Control emulsion.

From publications we know a series of data concerning the LD_{50} of DDT after injection in other animals and also in *Periplaneta*. The most important data are collected in TABLE IV, where in each case the publications in question are referred to. Moreover we have combined, from various publications, findings concerning the LD_{50} of DDT as a contact poison and when applied orally.

TABLE IV

LD_{50} of DDT in mg./kg. b.w. for various species.

Species	Skin application	Injection	Oral administration	Author
Periplaneta americana .	10—47	5—100	—	[73, 150, 172, 174]
Carausius morosus . .	—	60	—	[58]
Prodenia eridania . .	—	—	60	[197]
Musca domestica . . .	2—28	—	—	[48, 49, 92, 156, 170, 174]
Calliphora sp.	9—28	—	—	[174]
Aedes aegypti	5·5—8	—	—	[48, 49]
Melanoplus differentialis	(rather much)	—	50	[85]
Cimex lectularius . .	63	—	—	[24]
Pediculus humanus . .	27	—	—	[24]
Cambarus virilis . . .	—	0·5 (!)	—	[180]
Cancer irroratus . . .	—	< 0·03(!)	—	[179]
("Fish")	suscept. to highly dispersed suspensions	—	< 5 (!)	[41, 69]
Hen	pr.nt. [1])	—	300—500	[7, 26, 168]
Rat	300	40—1500	200—800	[7, 26, 27, 28, 51, 73, 129]
Mouse	pr.nt. [1])	—	450	[26]
Guinea pig	1000	900	400	[27, 28]
Rabbit	300—3000	25—250	300—1500	[7, 26, 27, 28, 51, 129]
Cat	pr.nt. [1])	25—40	250—400	[7, 129]
Dog	,,	60—75	600—750	[39, 129]
Horse	,,	—	260	[25, 168]
Goat	,,	—	6500	[168]
Monkey	,,	50—60	500—600	[25, 129]

[1]) pr.nt.: practically non-toxic.

It appears that our results in *Periplaneta* agree very nicely with those of other investigators. For the rest, it is remarkable that the LD_{50} of DDT for one species is often stated as varying widely. This is very largely due to differences in the liquids in which DDT was dissolved for the purpose of application.

There is no doubt that this dependence on the solvent is connected with the solubility of DDT and the way of resorption by the various tissues. To illustrate this we made a different arrangement of part of the data in TABLES V and VI.

TABLE V

Influence of the solvent on the LD_{50} of DDT.
LD_{50} in mg./kg. b.w. (Injections).

Species	Emulsion	Liquid paraffine	Oil	Acetone	Author
Periplaneta . .	18—35	—	82—100	5—8	[150, 172, 174]
Rat	30—50[1])	1500[2])	> 180[2])	—	[7, 26, 73, 129]
Rabbit . . .	35—50[1])	250[2])	—	—	[7, 27, 129]

[1]) Intravenous. [2]) Subcutaneous.

TABLE VI

Influence of the solvent on the LD_{50} of DDT.
LD_{50} in mg./kg. b.w. (Oral administration).

Species	Emulsion	Liquid paraffine	Oil	Cristalline DDT	Author
Rat	400—500	800	250	—	[27, 51, 129]
Rabbit . . .	350—500	400	1500	—	[27, 51, 129]
Guinea pig .	—	400	560	—	[26, 27]
Goat	—	—	—	> 6500	[168]
Hen	—	—	ca. 300	ca. 500	[26, 168]

Discussion

It is not difficult to explain our results and the findings furnished by the literature. It is very clear that DDT when injected is certainly not appreciably more toxic to insects than to other animals. Insects, however, can also very easily absorb a lethal dose through the skin, which is not the case in other animals (especially Vertebrates).

It goes without saying that for the explanation we think of the lipophilic property of DDT — a fact which has been mentioned by other authors [9, 94, 125, 141, 143, 144, 174] and by us [58].

The skin of a Vertebrate owing to its high water-content represents a physical barrier against the lipophilic DDT, whereas the insect cuticle, by its richness in lipoids actually forms a suitable entrance for this molecule (compare [92]). This view is corroborated by the fact that a medium with an affinity both to water and lipoids, for example acetone, can break up the water barrier, so that a DDT-acetone solution can indeed act as a contact poison to Vertebrates [34, 54, 191; cf. also 45]. In this connexion there have been warnings against the use of fatty ointments containing DDT, for example to combat scabies in man [4; cf. also 27, 33, 34]. DDT in fine colloidal dispersion in the surrounding water in concentrations varying from $1 : 10^4$ to $1 : 10^7$ is toxic to fishes. Coarser suspensions, however, are not very dangerous [69]. We see from this that the degree of dispersion in which DDT is applied also plays an important part in its penetration.

It appeared furthermore that not only insects but also other animals with a chitinous cuticle are susceptible to DDT as a contact poison. Very impressive in this connexion are investigations in which among other things it appeared that within the group of the Coelenterates species of the genera *Obelia* and *Campanularia* are susceptible to highly diluted suspensions in the surrounding water, whereas, for example, *Hydractinia* did not respond to it. As is known, the former possess a chitinous perisarc, whereas *Hydractinia* does not [144, 145]. The same author could also prove, that chitine was capable of concentrating DDT by adsorption from highly diluted aqueous suspensions. Some of these facts are clearly illustrated in TABLE VII.

That adsorption-processes are of great importance for the entrance of DDT through the skin, is also borne out by other investigators. The mortality of mosquitoes and bed bugs exposed to a DDT-residue (contact action) is greater at low temperatures than at high ones. In other words the contact action is governed by a negative temperature-coefficient [101, 141]. This has also been found to be the case in *Tribolium castanium* [132], while there were similar results in *Musca domestica* [92, 102]. The phenomenon was somewhat more closely studied in larvae of *Aedes* and *Chaoborus* [61, 143]. There it appeared that the temperature-coefficient is

positive at comparatively high concentrations of DDT in the surrounding water (suspensions), but negative at low concentrations.

Table VII

The LD50 of DDT, expressed in mg. DDT per l. of water, for various aquatic animals

Species	LD50	Author
Obelia sp.	0·1	[144]
Campanularia sp.	0·1	[144]
Tubularia sp.	1·0	[144]
Pennaria sp.	1·0	[144]
Hydractinia sp.	10·1	[144]
Astrangia sp.	10·0	[144]
Daphnia sp.	0·001	[2, 144]
Cyclops sp.	< 1·0	[143]
Gammarus sp.	0·5	[144]
Artemia salina	0·1	[144]
Pagurus longicarpus	0·5	[144]
(*Anopheles* sp., larva)	(0·005)	[148]

If the animals were for a short time kept in a diluted suspension at a low temperature, this pre-treatment accelerated the toxic action of a suspension at high temperature to which they were transferred afterwards. High-temperature pre-treatment, however, did not affect the toxic action of following treatment at low temperature. When the test animals were injected with DDT, the temperature-coefficient was always positive. These tests make it indeed highly probable, that adsorption by the skin plays an important role in the contact action of DDT.

Meanwhile it remains disputable in what way DDT once concentrated in the skin gets from there into the rest of the body. There are no clear data on this subject, although transport along the nerves is taken as being probable by some investigators [94].

We do not believe that this view is satisfactory in every respect. It is true that all sorts of fat-solvents, among which also well-known insecticides (e.g. kerosene), can be selectively accumulated in the insect nervous system [140, 146]. It remains, however, doubtful whether such facts may be connected with our problem. After all, we have to do here not with a liquid, but with a solid, which yet for its transport probably stands in need of a special lipophilic medium.

It is, therefore, not impossible that the substance dissolves in the lipoids of the haemolymph, and is thus carried to all parts of the body. In *Periplaneta* it was actually proved that the transport of DDT along a leg is not necessarily confined to an intact nerve, and that the exoskeleton is not required for it either [170]. So there are not many other possibilities left than a transport by the haemolymph. As yet nothing is known on this subject with any certainty.

Other experiments [58, 85, 156] brought to light that the insect cuticle responds similarly to various other contact insecticides, e.g. hexachlorocyclohexane, "Velsicol 1068", etc. In TABLE VIII a few facts concerning gamma-hexachlorocyclohexane are given for comparison. It is practically certain, that we are dealing here with a general property of the action of contact insecticides.

TABLE VIII

LD50 of γ-hexachlorocyclohexane for various species, expressed in mg./kg. b.w.

Species	Skin-application	Injection	Oral administration	Author
Periplaneta americana .	4—4·6	3·4—17	—	[58, 156, 170]
Musca domestica	0·3—3 dependent on age	—	—	[48, 49, 156, 170]
Calliphora sp.	0·6—1	—	—	[156, 170]
Anopheles sp., larvae . .	0·064[1])	—	—	[40]
Culex sp., larvae . . .	0·064[1])	—	—	[40]
Aedes aegypti	3—3·5	—	—	[48, 49]
Cimex lectularius . . .	6	—	—	[24]
Pediculus humanus . . .	1·5	—	—	[24]
Melanoplus differentialis	—	—	5—10	[85]
Rat	500	< 50[2])	200	[28]
Rabbit	300	< 75[2])	200	[28]
Guinea pig	400	< 100[2])	100	[28]
Mouse	300	—	—	[28]
Rana esculenta	—	< 50[2])	—	[58]

[1]) micrograms per animal. [2]) subcutaneous.

When we ask ourselves whether from the above a conclusion can be drawn with regard to our main problem, it appears that indeed

some progress has been made: a primary condition to the finding of new and more effective chemical contact insecticides is a high lipoid solubility of the "candidate insecticides". *Lipophobic contact insecticides* is evidently a contradiction in terms.

Review of the literature

So far we have examined the specific contact action by means of a comparison between the toxicity following skin application and following injection. We already mentioned that others also have examined the oral toxicity. Some of their findings are shown in TABLE IV. In this TABLE the data under the heading "Injection" are not only difficult to compare, owing to the influence of the solvent (P. 29), but also owing to the many different ways in which the injection has been effected.

There is, especially in Vertebrates, a marked difference in the action of a substance after subcutaneous, intravenous, intramuscular, or intraperitoneal injection, and there are some further possibilities.

On the oral toxicity of DDT and on the differences in action resulting from the various methods of injection we have made no experiments ourselves, but for the sake of completeness we present a short discussion of the results given in publications on the subject.

After our foregoing explanations it is to be expected that DDT, when applied orally, is not, or, at least, not very toxic. For, the intestinal wall, too, may be considered as a water barrier, so that resorption conditions seem *a priori* unfavourable. On the other hand it should be kept in mind that the intestine is none the less capable of absorbing fats, or rather fatty acids, possibly together with substances dissolved in them, while it is also conceivable that DDT by fermentative processes can be converted into a water-soluble compound. We may expect, therefore, that the oral toxicity will be intermediary between the contact toxicity and that following injection, or at most equivalent to the latter. About the oral toxicity in insects we can predict still less, because we know very little about the processes taking place in the intestinal tract of these animals. Yet it may be expected, that here the toxicity following oral administration will not be larger than that following injection.

A detailed discussion of the toxicity of DDT in Vertebrates following different ways of injection (see above) will not be given

here; this omission is of little consequence as in most cases the intravenous injection was applied. The question as to what form of injection in Vertebrates compares from a physiological standpoint best with the intra-abdominal injection applied in *Periplaneta* (and other insects) is important. We are of opinion, that the intravenous injection answers the purpose best, because here, as well as in the intra-abdominal injection, an immediate contact between the blood (or haemolymph) and the injection-liquid is ensured [1]).

We shall now enter into a somewhat closer examination of the oral toxicity of DDT. As it is of practical importance that there are reliable data on this subject, it has already been extensively studied. The following survey can on no account claim to be exhaustive, but we do not wish to omit a general outline.

The oral toxicity of DDT in man is a matter of controversy, but we may assume that the practical use of DDT offers no danger of acute poisoning (except, of course, in the case of gross imprudence). Chronic poisoning is possible, but this we leave out of consideration [2]).

Some fatal cases of acute intoxication in man are described. But these were always cases of accident [15, 80]. The lethal dose according to these data is in the neighbourhood of 150 mg./kg. b.w., though opinions differ as to the part played in these cases by the solvent [14, 79].

On the other hand we know of cases in which volunteers took DDT *per os* without any toxic effect. The doses here, however, were comparatively small (6 to 11 mg./kg. b.w.) [11, 119]. More curious is a report in the British Medical Journal [6], according to which a venturesome person survived the eating of a large quantity of pancakes made of DDT-powder instead of flour. It is likely that DDT, prior to consumption, was converted by the baking-process into some other non-toxic compounds, as it is known that DDT is easily decomposed at high temperatures [56].

In the case of a man who had chewed tobacco in which there was 5 per cent DDT, violent pains occurred, accompanied by other

---

[1]) In our own experiments we always injected *Rana*, for technical reasons, into the lymph sac. This classic method has always been satisfactory, and so it was here.

[2]) In this connexion the limit of tolerance for DDT-residues on foodstuffs in America is 7 mg. per kg. [12, 50]. DDT-treatment of cheese (against *Piophila* sp.) is disencouraged [3], etc. [cf. also 10, 128].

symptoms. These symptoms continued for hours, but in the end he recovered spontaneously [161]. Generally, rather serious symptoms may occur after doses lying far below the lethal dose [84].

We shall not give a detailed account of the oral toxicity of DDT in various Vertebrates. A glance at the TABLES IV and VI shows that here the LD50 is indeed mostly much higher than the LD50 following injection [cf. 5, 25, 41]. The absorption of DDT-crystals through the intestine in Vertebrates is difficult; solutions, for example in oil, are, however, absorbed [11, 36, 126, 194].

Many experimental data are available concerning chronic poisoning in all kinds of animals, after oral administration of sublethal doses, but they will not be discussed here [cf. 27, 32, 36, 50, 62, 84, 126, 193].

It need not be stressed that investigations about the action of DDT as a "stomach poison" in pest insects is for practical purposes of secondary importance. It is a pity, however, that owing to this circumstance the available data concerning the action of DDT as a stomach poison are so scanty and unreliable that they shed very little light on the part played by the intestinal tract in the process of poisoning.

Locusts, which remarkably enough are not very susceptible to DDT as a contact poison, die after eating it; the LD50 for *Melanoplus differentialis*, for example, is 50 γ/g. b.w. [85]. Also in other species (*M. mexicanus*, *M. bivittatus*, *M. femur-rubrum* and others) DDT acted as a stomach poison [82, 192].

Bombyx mori (larvae) and *Epicauta atomaria* are killed by about 35 γ per animal [96]. Bees are killed by a concentration of 0·05 per cent DDT in their food [83] [cf. 57].

Periplaneta, however, can live for three weeks on food containing 1 per cent of DDT. Here death occurs later than after contact poisoning [117]. In the case of flies and moths there is also information available concerning the action of DDT as a stomach poison [164, 167], as is also the case in *Mamestra* and *Pieris* [109].

Interesting in this context are experiments in which it was tried to kill blood-sucking parasites by introducing the insecticide, either directly or via the intestine, into the host's blood. Blood of rabbits that had received about 400 mg. DDT/kg. b.w. in their food, proved mortal to *Cimex lectularius* and *C. hemipterus* [100]. Similar results were obtained with γ-hexachlorocyclohexane [112]. These investig-

ations are still at an early stage. Perhaps they will yield their fruits in due time.

This survey confirms the view that DDT is a universal poison (at any rate in the animal kingdom) and that its specific contact action in Arthropodes results from its physical properties in connexion with the properties of their skin. None the less there are considerable differences in susceptibility within the group of Arthropodes. Compare for example the LD50 for *Cancer irroratus* with that for *Periplaneta* (TABLE IV). It would be very interesting in this connexion, if the toxicity of DDT after injection were determined for Aphids, as it is generally claimed, that these animals are highly resistant to DDT as a contact poison [cf. 57, 184].

After our foregoing treatment of the matter the absorption of DDT via the respiratory tract, which in the case of mammals is possible, for example by a stay in an aerosol containing DDT, does not offer us any new problems of principle [cf. 120].

As we consider the first question to be sufficiently dealt with for our own purposes, both by the data furnished by the literature on the subject and by our own supplementary experiments, we may now conclude this chapter. Before starting the discussion of our second problem (CHAPTER III) we will first go a little deeper into the question of the intermediary metabolism of DDT.

Chapter II

INTERMEDIARY METABOLISM OF DDT

Introduction

We had reason to suppose that the processes we are now going to discuss are not directly linked with our problem, and therefore we did not attempt to adduce any fresh experimental evidence. This chapter thus falls somewhat outside our particular field of research. Yet, since the physiologist should at least have a general knowledge of this aspect of the DDT-problem, we shall cast a cursory glance over the rather extensive literature on the subject.

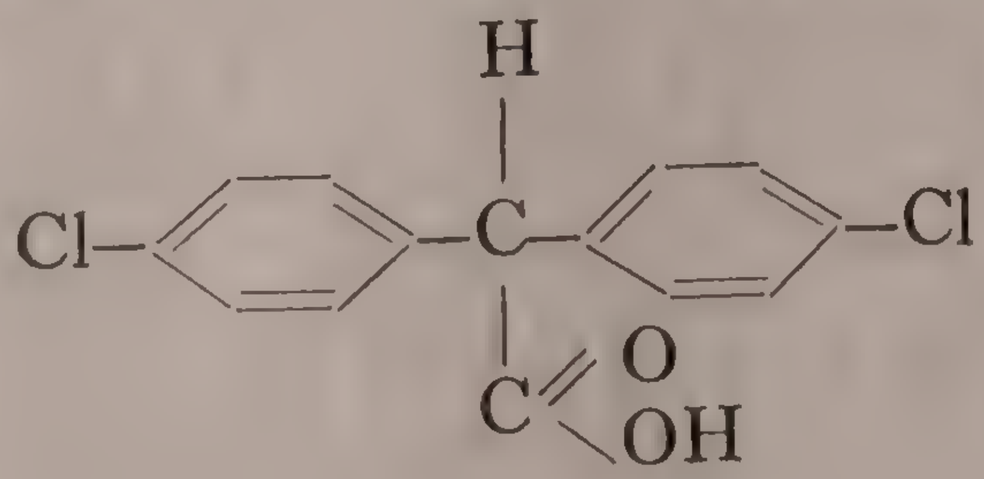
The question we seek to answer runs somewhat like this: "In what manner does the "defense-mechanism" of the body react to the foreign DDT-molecule?" By "defense-mechanism" we mean for the time being nothing more than the intermediary metabolism of DDT, i.e. the processes of detoxification and excretion.

It is possible that a storage of DDT takes place in such a way that, in spite of its presence, it remains harmless to the organism. Little is as yet known about all this; and our knowledge is almost exclusively limited to the physiology of mammals. As to what happens in the case of insects we have hardly any information at all.

Excretion-products after DDT poisoning

Urine and bile

In the urine of all sorts of laboratory animals after DDT poisoning a metabolite of DDT is found which is generally indicated by "DDA". It is the carboxylic acid derivative of DDT, viz. the water-soluble compound 2,2-bis(p-chlorophenyl)-acetic-acid,



This compound was found in the urine of rabbits, rats, cats, dogs and other animals [11, 25, 36, 124, 125, 163, 166, 185, 186, 187].

Also in man this excretion-product was found in the urine after DDT poisoning (or DDT absorption) [11, 119, 161].

Owing to the presence of this compound the *p*H of the urine sometimes decreased from 7·5 to 6 or 5 [34].

It is not always easy to determine DDA quantitatively by chemical analysis, but several methods of determination have been elaborated (including also physical ones), to which we have referred already in another publication [56] [cf. 122, 185].

DDA is not the only excretion-product characteristic of DDT poisoning. Unchanged DDT can also be present in the urine, sometimes in still greater quantities than DDA. There is no agreement on this point. For example, in man some investigators found that three times as much DDT was excreted as DDA [161]; others found exclusively DDA, and not a trace of DDT [119]. In this respect there also seems to be a wide divergence between different species of animals. Some investigators found no DDT in the urine of their test animals (e.g. rabbits), but found DDA accompanied by two neutral DDT-derivatives [25]. They are probably esters of DDA, as after hydrolysis with acids or alkaline DDA is yielded. They occur for example in a ratio of one to three DDA in urine [25, 125, 166].

The question arises whether DDA retains the toxic action of DDT or whether we have to do here with a (or *the*?) final product of the process of detoxification. Although it seems very simple to solve this question by means of injecting DDA in test animals, yet this has so far been done in an astonishingly small number of cases. In cats an intravenous injection with 25 to 75 mg. DDA per kg. b.w. did not produce any symptoms of poisoning [36, 147]. In the case of rabbits DDA is said to have only one fourth to one fifth of the toxicity of DDT, besides producing a different pattern of symptoms [11]. In dogs DDA administered orally could not call forth any symptoms of poisoning that are worth mentioning [25]. Finally some information is available about the toxicity of DDA to Everte-brates. The LD_{50} of DDA as a contact poison for *Musca domestica* is stated by some as 500 γ/g. b.w. [92], by others as even more than 1300 γ/g. b.w. [170].

Considering what was said in CHAPTER I (p. 30) concerning the influence of the lipoid-solubility on contact insecticidal action, these

figures hardly contribute to the solution of our particular problem.

The fact that an intra-abdominal injection of DDA in *Periplaneta americana* does not have the least toxic effect is of greater relevance [170]. Moreover, whereas DDT is said to act specifically on the peripheral nerves and a little less so on the heart of the crab, this was not found to be the case for DDA [179, 180].

But, to our regret we do not yet know whether DDA occurs actually as an excretion-product after DDT poisoning in Everte-brates. So long as this remains unknown, little can be concluded from these facts.

DDA is also found as an excretion-product in the urine after poiso-ning with "DDD" [1]) or after administration of the DDT-ole-fine [2]) [25].

By the catalytic action of anorganic chlorides, or by hydrolysis with alcoholic alkaline, DDT *in vitro* can easily be de-hydrohalog-enized into the corresponding olefine [65, 177], which, as we just have seen, can in its turn be converted into DDA in the organism. In how far these two facts may be of significance for understanding the processes that eventually lead to the excretion of DDA, cannot yet be said. In this context, however, we wish to point out that the olefine is *in vitro* rather resistant to hydrolysis [177] so that it is not very likely that the reaction

$$\overset{\|}{C}Cl_2 \rightarrow \overset{|}{C}\begin{matrix} =O \\ -OH \end{matrix}$$

actually occurs in the organism.

In view of what has just been said, it is of no great interest to know that it is possible to prove the presence of DDA in the kidneys. What matters more is that this excretion-product is also found in the liver [25, 163]. This has led some investigators to the assumption that the hydrolysis of DDT into DDA takes place in the liver [25].

Although such a hypothesis looks attractive, an extensive investiga-tion is required before we can accept it. For the rest, there are some other facts that may be adduced.

In the first place it is known that after chronic DDT poisoning there always occur degenerative changes in the histological structure of the liver (and the kidney). This has been observed amongst other

[1]) 2,2-bis(p-chlorophenyl)-1,1-dichloroethane.

[2]) 2,2-bis(p-chlorophenyl)-1,1-dichloroethylene.

animals in rats, mice, rabbits, guinea-pigs, cats, dogs and monkeys [7, 25, 27, 39, 120, 147]. Usually we find the picture of a centrolobular fatty degeneration and a necrosis, although this picture varies according to the species considered [163]. According to some investigators these injuries of the liver are so serious, that they may be considered as the cause of death in DDT poisoning [26]. It is true that pathological changes of this kind are not specific for DDT poisoning, as also other poisons are in the long run detrimental to the liver and the kidneys, but this is not to the point in our special problem. At any rate, that "something happens" with DDT in the liver is rather likely.

Secondly the fact that in the liver relatively little DDT is found [37, 50] may support the above-mentioned view. For, if DDT is converted in the liver into DDA, while this metabolite is again passed back to the blood, this "va et vient" will render an accumulation in the liver improbable. From tests in which the fat-content of the liver was varied by certain diets, it appeared that its DDT-content was higher in the case of a high fat-content than of a low one [38]. But this does not surprise us, as DDT is accumulated in all fat-depots of the body (see below).

In the third place we mention that in rabbits DDT or DDA (not further examined) was also found in the bile [163].

So we see that there is as yet little support to be had from the available experimental data. Yet they give us an indication of the direction in which research in this field may develop. What we need in the first place is a further analysis of the action of DDA, when it is brought into the organism. Secondly the influence of the liver on the intermediary metabolism should be further examined.

Milk

It is common knowledge that the mammae of lactating mammals with regard to various substances can behave as excretory organs. This raises the question whether perhaps after DDT poisoning DDT or related compounds may appear in the milk. That this question concerns not only the physiologist, but has also great practical importance, calls for no detailed explanation. Dairy-cattle, for example, are perpetually in danger of taking up DDT with their food (e.g. when they are fed on DDT-treated plants).

It appears indeed from a great number of experiments that the milk of all sorts of mammals after oral DDT-administration contains DDT. This was found for example in the milk of dogs [25, 194], goats [169] and cows [123, 149]. Young rats died after typical DDT symptoms, when they were suckled by a mother-animal that received 1 per cent of DDT in her food. A case has even been reported in which DDT after passing two hosts still found its way in toxic quantities into the milk: a goat was given DDT *per os*; on the milk of this animal a lactating rat was fed. And the rat's young died of DDT poisoning.

It is especially in the milk-fats, in the cream therefore, that we find DDT concentrated, which is not surprising, seeing that it dissolves so much more easily in fats than in water [169].

Nowhere did we find indications of anything other than DDT appearing in the milk. DDA, for example, has never been found. So it is very likely that after absorption there is no quantitative conversion of DDT into DDA. In some way or other, probably dissolved in the lipoids of the plasma a part keeps circulating in the blood. In rabbits this was proved directly [163], while the excretion of unchanged DDT in the urine of several other animals points in the same direction.

Faeces

Though it is hardly admissible to consider the faeces as excretion products in the strict sense of the word, we shall yet treat under this heading what is known about the occurrence of DDT in the faeces.

As we saw, DDT is absorbed by the intestine with great difficulty. This appeared probable in view of its slight oral toxicity. But this is no proof. *A priori* it is very well conceivable, that the DDT-molecule in the intestinal tract undergoes such changes that it loses its toxicity, while there is also the possibility of detoxification by the liver.

Now we saw that there are indeed arguments in favour of the latter possibility, but this process does not give us a complete explanation of the high LD_{50} of DDT administered orally. For if the ingested quantity of DDT is compared with the excreted quantity of DDT and DDA in the urine, it turns out that a high percentage remains unaccounted for. This part must either be left behind in the

organism or must have left it by another way [1]). Both possibilities are realized. About the DDT retained in the organism we shall speak below.

There appears indeed a great deal of the ingested DDT unchanged in the faeces [165]. The percentage arriving there is, in proportion to the part excreted by the kidney, higher according as the dose taken was higher [163]. In rabbits 80 to 90 per cent of the total excreted DDT can occur in the faeces [124]. From which it appears that this compound is not materially affected by the digestive enzymes and that the resorption by the intestinal wall is far from complete.

Although this is no absolute proof that the DDT occurring in the faeces is indeed unabsorbed material, we may yet safely assume that it is. For it is unlikely that such great quantities of the substance should be absorbed in the blood, to be afterwards excreted with the bile.

Storage of DDT

It was pointed out already that part of the absorbed DDT is retained by the organism. Quantities show wide variation from one species to another but retention has been observed in all animals so far examined.

The principal depot is the fat-tissue. In pigs, for example, after chronic oral administration of DDT, quantities of 250 to 350 mg./kg. were found in the fat [50], at a moment when the animal did not yet show any signs of poisoning. In cows something similar was found: here the fat-tissue contained 150 mg./kg., whereas in other tissues and organs 1 to 7 mg./kg. was found [149]. From this we learn at the same time that the accumulation of DDT can also be applied by the organism as a defence mechanism.

In the fat-tissue of various laboratory animals great quantities of DDT also appear to be stored, e.g. in rabbits, rats, cats and other animals [11, 25, 37, 104, 124, 125, 163]. In rats the perirenal fat-tissue after a single oral dose of DDT of about 1 mg./kg. b.w. contained a relative quantity of DDT about 3700 times larger [25].

Sometimes it is pointed out that in other tissues and organs a proportionally great quantity of DDT is stored, e.g. in the spleen, the spinal cord [25] and the adrenal glands [37, 95, 104]. Among

[1]) For it is not likely that DDT is broken down to quite different molecules as it is very stable. Moreover, such a conversion has never been found.

these data, however, there is far less agreement than among those bearing on the role of the fat-depots [cf. 149].

The above data are certainly connected with the fact that often days or weeks after acute DDT poisoning DDT may still be found in the urine or the milk of test animals [11, 123, 163] [1]).

Discussion

We have now some rough idea how the absorbed poison may find its way into the excretion-products. DDT, probably dissolved in the lipoids of the plasma, is transported to the fat-depots of the organism, and there for the greater part retained, while a relatively small part of the total amount keeps circulating in the blood.

In the liver it is partly hydrolysed into DDA, and another part is excreted as DDT with the bile. In the kidney DDA as well as unchanged DDT are withdrawn from the blood and excreted in the urine.

It was found in dogs, that DDT does not remain in the fat-depots, but that it gradually disappears from them when the DDT is no longer being administered [194].

Meanwhile part of the substance will find its way to those organs, where in virtue of its toxic action it can produce the specific DDT-effect.

In order to learn something more about the movements of the DDT-molecule in the insect-organism, attempts have been made to trace the molecule.

This method, by which stable or radio-active isotopes are introduced into the molecule, is of ever-increasing importance in physiological research.

To this purpose the bromine-analogue of DDT was synthesized i.e. 2,2-bis-(p-bromophenyl)-1,1,1-trichloroethane, in which the radio-active isotope Br^{82} (half-life time 34h) was used. From other investigations it was already known, that the behaviour of "Br-DDT" in the organism is similar to that of DDT. Its toxicity is slightly inferior, but the symptoms are of the same nature, also in mammals [particularly 25, 74].

Thus it was hoped to find out something more about the places where DDT is ultimately deposed, but so far these efforts have failed. The first endeavour in this field [74] gave as the only result

[1]) The fact that such a large storage of DDT can occur in fatty tissues is of course of practical importance in connexion with the consumption of animal fats.

that radio-active Br was, finally, found in all parts of the organism. In these experiments the compound was injected in *Periplaneta americana* and *Galleria mellonella*. Later it was observed in *Periplaneta* that a fair quantity of this "Br-DDT" found its way to the cerci and the ovaries, while less was found in the antennae, the testes, the trachea, the thoracic muscles, the salivary glands and the central nervous system.

More interesting is that in the Malpighian tubules or in the fat-tissue no trace of it was found [42] [cf. also 9]. With respect to this it is worth mentioning, that in a histological examination of *Periplaneta* after DDT poisoning no pathological changes were found in the Malpighian tubules [142].

Yet we must admit that our positive knowledge of the processes of intermediary metabolism of DDT in insects is practically nil. The mentioned facts only stress our ignorance; further research into these problems would certainly be interesting and might be elucidative.

Although we have not referred to the entire literature on the problem of the intermediary metabolism of DDT [cf. 90, 91, 162], we have so much widened our horizon, that we can now continue the discussion of our own research.

Chapter III

GENERAL DISCUSSION OF THE DDT SYMPTOMS AND THEIR CAUSES

Introduction

We must now deal with the problem of how DDT, once it has reached the internal environment of the organism, manifests its toxic action. Contrary to the problem we discussed in Chapter I, for which the findings of various investigators brought a speedy solution, the evidence on this subject has so far been heterogeneous and contradictory. It stands to reason that such a mass of experimental data has given rise to contradictory explanations. In the following we shall consider the most important views, but it seems expedient that we first have a closer look at the actual DDT symptoms.

DDT symptoms

A short time after acute DDT poisoning the animals — insects as well as other animals and man [80, 81, 127, 191] — become uneasy, and excited. They show a hypersensitivity to external stimuli.

Gradually they pass from this first stage [1]) into a period in which they show sudden outbursts of motor activity. We observe violent tremors, relieved by periods of seeming tranquillity. Often they look as if they were cold and are shivering. These tremors become more and more violent and frequent. This second stage can continue for a period stretching from a few hours to a few days, depending on the species.

In the third stage the animals lose the ability to maintain their normal position. They fall down or topple over, they make random movements and besides the tremors violent spasms and convulsions appear. In this stage it is not likely that the sense of equilibrium itself is affected, as the animals evidently strain to recover their

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[1]) For convenience' sake we shall indicate the successive stages of poisoning as first, second, etc. Of course there is a gradual transition from one stage into another.

normal position, in which however they do not succeed owing to their hyper motor activity.

Gradually this stage passes into the last one (the 4th), in which the tremors and spasms subside in violence and frequency, until ultimatively they disappear altogether, and complete immobility results. We never found the toxic action to be reversible in this fourth stage. But it is not easy to prove that recovery is absolutely impossible. Exceptionally others have indeed observed a recovery, e.g. in bees [63]. In *Rana*, for example, the heart continues to beat for hours after the beginning of the fourth stage, while also other functions proceed normally, to which phenomena we shall return later. We regard these data, however, with some reserve [cf. 157].

Death follows, at some moment after the rise of this fourth stage. When exactly this occurs is just as difficult to determine as the meaning of the word "death". In our experiments we called an animal dead, when it lay entirely limp and motionless and no longer responded to rough mechanical stimulation. Mammals die already in the third stage, but this difference results from secondary causes, which will be discussed in due course.

It is true, that by the above the DDT symptoms are not described exhaustively, but it is sufficient to have become acquainted with their main features. What matters, after all, is to understand the cause of these very striking phenomena. In the following we shall have occasion to give some additional information.

Survey of relevant literature

It is anything but easy to follow a logically justified course in reviewing the literature in question. The problem has been tackled from multifareous points of view, and the result is a mass of heterogeneous facts. We beg the reader's indulgence, if in our effort to follow a straight path, we should occasionally be carried away by the general confusion.

A number of investigators asked themselves whether perhaps DDT poisoning would occasion such morphological and histological changes that from them some insight into its action might be obtained. Others again wondered if a closer study of the DDT poisoned animal's metabolism might shed some light on the problem. In this connexion attention was also paid to the water balance, the role of

ions etc. Another very large field of investigation is closely linked with this, viz. the question about the relation between toxicity on the one hand and chemical structure and physical properties on the other. Also the question whether DDT perhaps has a general action on the protoplasm or affects growth and development, was by some chosen as a starting-point for their experiments.

In view of these different approaches we shall divide the matter to be reviewed into the following sections:

1. Histological changes after DDT poisoning.
2. Influence of DDT on metabolism.
3. Relation between toxicity and chemical or physical properties.
4. Influence of DDT on living matter in general.

In many cases the investigation was concerned with phenomena that show up clearly only after chronic poisoning. Our attention being specially riveted on acute DDT poisoning, we shall not enter into a detailed discussion of these problems. Yet it seems expedient not to omit this large field of research altogether.

The action of DDT on the nervous system has been studied very extensively. As this research is closely linked with our own work, we shall not anticipate it here. In Chapter VI we shall have occasion to thoroughly go into it, while in treating our own work we shall time and again introduce subjects connected with it.

It is to be noted forthwith that not all the investigations to be treated below were made with the special intention of finding an explanation for DDT symptoms. Yet we think they ought to be reviewed here, as they form an important contribution towards our general understanding of the physiological action of DDT. Such understanding is an indispensable weapon to attack our own problem succesfully.

Histological changes after DDT poisoning.

It will be clear that after acute DDT poisoning we may hardly expect any morphological or histological changes. In the following, therefore, we shall mainly have to do with chronic absorption of sublethal doses, *per os*, through the skin, or otherwise.

Mammals.

Liver, kidney and intestinal tract. The histological changes we see

appear in the liver and also in the kidney in mammals after DDT poisoning, were already treated in another context (p. 40). It is superfluous to discuss these facts once more, but one remark should be made. It would be wrong to interpret these changes as the cause of the typical DDT symptoms. In the first place it is hard to understand how for example fatty degeneration of the liver could be the cause of spasms and tremors. Furthermore it is known that DDT symptoms also become manifest before there are any changes in the liver. And thirdly eviscerated cats were observed in which the DDT symptoms continued undisturbedly [7, 11].

It follows that the liver, degenerated or not, cannot be responsible for these symptoms.

The latter experiment excludes also the possibility that any other part of the intestinal tract should play a leading part in the production of DDT symptoms. Thus the irritating action of DDT (*per os*) on parts of the intestine (especially on the pylorus and the duodenum) observed by some investigators [39] cannot have any essential meaning [cf. also 126].

Blood and circulatory system. In a number of cases irregularities were found in the blood picture of DDT-animals [26, 27, 125]. Such irregularities, however, do not invariably accompany DDT poisoning. Rabbits, after fair oral doses of DDT did not show any deviations of the normal haemoglobin-content, while also the number of erythrocytes, and the number and type of leucocytes remained unchanged [51].

In man it was sometimes found that upon DDT absorption changes in the blood picture and in the blood pressure occurred [34]. Blood pressure also changed in some laboratory animals [36, 125].

Histo-pathological changes of the cardiac muscle were observed in a few cases, but we cannot escape the impression that these were non-specific [cf. 11, 25, 27]. In dogs, after oral administration of "DDD" moderate pathological changes of the heart were observed [53].

Nervous and muscular system. The nature of the DDT symptoms points to a specific action on the nervous system and, less clearly, on the muscular system. In this connexion an extensive search has

been made for any histo-pathological changes in the nervous and muscular systems.

Slight aberrations were found in the staining of preparations of the myoneural junctions (in rats), but these did not admit of any clear interpretation [29]. The histological picture of voluntary muscles after DDT poisoning rarely if ever differs from the normal preparation [25].

Conspicuous histological degeneration was, however, often found in the central nervous system. The most striking ones were found in the cerebellum, mainly in the *nucleus dentatus* and the cortex cells. Among other things an increase of the neuroglia and a necrotic degeneration and resorption of ganglionic cells was found. The Purkinje cells were less seriously affected than the other neurons [7, 11, 125]. Also in the spinal cord abnormalities of a degenerative nature were found [7, 27, 120].

So we find that especially the cerebellum and the spinal cord are histologically affected by DDT. Later we shall consider in how far these facts can be assimilated to a more general view, but we emphasize in anticipation that we cannot attach much value to such data. In the first place such changes were not found invariably, while there is neither an obvious relation between the size and spreading of the lesion and the quantity of DDT applied [7]. Secondly information of adequate precision about the nature of the anomalities is lacking.

The essentials of what is known about the occurrence of histological changes after DDT poisoning in mammals have now been sufficiently discussed. It is true that also in the adrenal glands, the thyroid and the parathyroid glands [25, 27] changes were sometimes found, while also skin irritations are mentioned, but these points may be left out of account [cf. 121].

It might be remarked that especially any change in the parathyroid glands could be of extreme importance, since these glands by their control of the calcium balance could have a great influence on the excitability of the (peripheral) nervous system. We quite agree with this remark, but the experimental data about the influence of DDT on the parathyroid glands are so scanty, that any further discussion of the subject must needs remain completely speculative. More extensive research in this field would certainly be interesting.

Insects.

It is strange that so little research has been made into the influence of DDT on the finer structure of the organs in insects. In the literature on the subject we cannot find one single positive result. Generally speaking, DDT does not seem to influence the histological structures to any high degree [cf. 70, 77]. In *Periplaneta* after poisoning no changes could be observed in the nerves, the Malpighian tubules, the nephrocytes, the heart and the muscles [142].

Our survey justifies the negative conclusion that we should not look for the solution of our problem in this direction. Now that it turns out that we do not get any further by only regarding the influence of DDT on the histological structure of the organs, we shall have to occupy ourselves with the possible influence on the functions of those organs.

Influence of DDT on metabolism

The fact that the metabolism after DDT poisoning is in many respects different from that of the normal animal, is not interesting in itself. We could hardly expect anything else. Yet it is conceivable that some of the differences will appear to be responsible for the typical DDT symptoms. It will not always be easy clearly to distinguish between primary and secondary metabolic changes that may occur after poisoning. This point should be kept in mind when considering the following facts.

Mammals.

The rate of basic-metabolism of DDT-animals is mostly higher than normal. This is always true for animals in a stage of acute poisoning, but it may also be the case in the less serious stages of chronic poisoning [7]. In rats an increase of more than 30 per cent above normal may occur [11, 125, 147], according to some it may even become twice as high as normal [36]. We are of opinion that such widely varying statements can be made, when in these measurings no due allowance is made for the influence of the tremors and spasms. Unfortunately we do not always find clear information about such technical details.

DDT poisoning may considerably raise the body-temperature.

In cats a few cases were encountered in which the animals died as a consequence of violent fever [36].

The influence of DDT on the tissue respiration of various organs has been very carefully studied. The oxygen-consumption of liver-slices (WARBURG determination) has been extensively studied. Generally an increase of the oxygen-consumption (up to about 25 per cent above the normal rate) is found, but not in cases of acute poisoning [147]. Only after the lapse of one to two days can the increased oxygen-consumption be demonstrated; and just at that time the first histological changes are to be seen.

We cannot yet give an opinion about the question whether this is mere coincidence, or whether there is a causal connexion between the two phenomena. At any rate it does not astonish us that DDT causes these changes in the liver, since the liver plays an important part in the detoxification of many poisons. Such a detoxifying action of the liver might well stimulate the metabolism and at the same time be detrimental to the liver cells themselves. Relevant information is as yet too scanty to decide the question.

In a later stage the oxygen-consumption of liver-slices becomes less than normal. Such investigations were especially made with liver-slices of rats, while different compounds were added as substrates [7, 11, 25, 36, 72, 73, 125, 147].

Similar measurements were made on other tissues (mostly of rats), e.g. brains, kidneys, and cardiac muscle. It is remarkable that in no organ was such a distinct influence of DDT found as in the liver [36, 72, 147], although also in other organs some increase in oxygen-consumption could be observed. It seems that the metabolism of muscular tissue of *Rana* is only increased after serious poisoning [36, 147].

In connexion with what has been said it will be understood that animals suffering from DDT poisoning are more susceptible to oxygen-shortage than normal ones [11].

The blood-sugar level of DDT-animals is subject to great changes, and this is especially the case after chronic poisoning. In dogs an acute DDT poisoning had no marked influence on the blood-sugar level [172]. Even comparatively small doses ultimately cause a large-scale mobilisation of liver-glycogen, which for example in rabbits produces a hyperglycaemia, in which the blood-sugar can attain

values up to 200 mg. per cent. Glucosuria, however, was never found.

Within a couple of hours the whole glycogen-reserve disappeared from the liver of a rabbit which had received 1 g. DDT per k.g. b.w. [95]. Also in rats after DDT poisoning a great decrease of the liver-glycogen was found [73, 125]. During and after the hyperglycaemic stage a large quantity of blood-sugar is immediately utilised in the muscles, as a result of their increased motor activity. Finally the blood-sugar level falls even below normal. In rabbits for example hypoglycaemia occurred, in which 20 to 30 mg. per cent of glucose was found in the blood. Some animals even died in consequence of a hypoglycaemic coma [95].

Administration of glucose may in such cases have a therapeutic effect. Rabbits after receiving a dose of DDT that was certainly lethal could be kept alive by injecting glucose.

Adrenalin-injections may in some cases have a therapeutic effect, but the action is only feeble and of a temporary nature. This is easy to understand, since the liver (and muscle) glycogen becomes exhausted: an order for mobilization has little sense when there are no soldiers left [139].

Also in cats an injection of glucose or Ca-gluconate acts therapeutically. The treatment should be repeated several times [36]. In dogs an injection of Ca-gluconate is said to be even a preventive against DDT poisoning [176]. We shall revert to this problem later.

A fall of the blood-sugar level is attended with a rise in the lactic acid content. We may safely assume that this is a consequence of the great muscular activity. In this process the *p*H of the blood remains constant: only a compensated acidosis occurs [95, 139, and others].

About the water and ion balance after DDT poisoning far less is known. From the available data it does not appear that this poison has an important influence on these functions. Increased water-absorption in DDT-animals has indeed been found, but this is attended with an increased uptake of food [36, 147], while the function of the kidneys remains normal until later stages [125]. In this context we may dismiss the fact that after chronic poisoning e.g. of dogs and rabbits, proteins were found in the urine [39], which is presumably connected with the histological degeneration symptoms mentioned before.

A distinct, though slight decrease in weight was found in various test animals (e.g. dogs and rats) after DDT administration [36, 147].

This does not make us much wiser, since all sorts of poisons may have a similar secondary action.

The alkali reserve of the blood decreases (rabbit) but the *p*H remains normal (cf. p. 52) [139]. After acute poisoning in dogs no changes could be found in the Ca, Mg, K, Na, Cl, and P-content of the blood [172]. Findings about the Ca-ions in the blood are very contradictory.

According to some investigators DDT poisoning would primarily cause a hypocalcaemia, which in its turn would be responsible for the symptoms. In favour of this hypothesis is the fact that Ca-gluconate, injected by way of preventive, can indeed suppress DDT symptoms [176], while $CaCl_2$ is also recommended as an antidote [139]. It should be noted, however, that mere glucose sufficed to produce a therapeutic effect [36, 139], and that others found the blood calcium actually to be increased instead of decreased [27]. It can also happen that the blood calcium level remains constant [37] and that $CaCl_2$ administration has no influence on the symptoms [36]. On the other hand we must mention that the administration of $CaCl_2$ or also of parathormone in some cases even accelerated the development of DDT symptoms [11].

These facts show that the role of the calcium-ion in the development of DDT symptoms is not yet clear. But the available information is still of such a nature as to compel us to take serious notice of this point, and further research is certainly to be recommended.

The findings concerning the therapeutic action of glucose and of calcium are confusing (e.g. in the case of injections with Ca-gluconate). On the one hand there is the influence of the glucose which can hardly be otherwise interpreted than as being a correction, beneficial to the animal, of the blood-sugar level; on the other hand there is the possibility that calcium, by its specific action on the nervous system affects the DDT symptoms.

Herewith we have sufficiently discussed the literature on the subject, although we did not review all investigations [cf. e.g. 99, 162].

Insects.

Comparatively few species have been investigated with a view to metabolism following DDT poisoning. This is due to the fact that,

on the one hand, most insects are for various reasons unsuitable test animals, and that, on the other hand, attention has been more focused on the action of DDT on mammals, in view of the potential dangers in practical applications.

In insects after DDT poisoning, just as in mammals, a more or less notable increase in oxygen-consumption was found. This increase may lead to 3 to 4 times the normal consumption, which was found, e.g. in *Popillia japonica* [105], *Musca domestica* [11] and *Tenebrio molitor* [72]. In other species the increase is smaller, e.g. in *Tribolium confusum* [72]. In some cases it could be demonstrated that the increased oxygen-consumption resulted from the violent muscular activity and was thus a secondary effect of the poisoning.

To this end DDT-animals (*Tenebrio*, *Periplaneta*, and others) were held for some time under narcosis. It appeared that the oxygen-consumption during that period was not higher than in normal animals, while after the narcosis they were yet in the same stage of poisoning as the controls (with DDT, without narcosis) [73, 115]. The specific, primary action itself was therefore not affected by the narcosis.

The increased rate of metabolism is of course also reflected by decreases in the carbohydrate reserve and body fat of the animals. Owing to violent muscular activity more than 90 per cent of the glycogen-reserve may disappear [105, 106, 115, 170]. The same holds for glucose. Body fats are drawn upon later. In *Periplaneta* for example no significant change in the fat content could be found after 48 hours [170], in *Chortophaga viridifasciata* (a locust) something similar was found.

That these phenomena are indeed secondary ones caused by the muscular activity, and are no lethal factor in themselves, appears not only from the above narcosis-experiments, but also from the fact that no therapeutic effect can be obtained by glucose-injections. So, contrary to what we observed in mammals, the carbohydrate deficit itself is here never the cause of death. This is not so strange when it is remembered that insects, thanks to their poikilothermia can bear a far wider variation in their metabolic rate than the homoiothermic mammals.

Furthermore the haemolymph in *Periplaneta* showed an increase (of approx. 70 per cent) in ketones and aldehydes. But this increase cannot be a cause of death either, as injection-tests showed that at

least 1000 times the normal quantity of such compounds was required to kill the animals [115].

In *Periplaneta* the body weight and the water-content are not seriously affected by the poisoning [115, 170, 172]. Imagines of *Popillia japonica* lose about 18 per cent of their water-content, their larvae probably nothing at all. It does seem though, that *Popillia* (larva, pupa and imago) after DDT poisoning desiccates sooner in dry air than it does normally [105, 106].

Hardly anything is known, in the case of insects, of a possible relation between DDT poisoning and the influence of certain ions. The only known fact is that calcium, administered as $CaCl_2$ (50 γ/g. b.w.) prior to a DDT-injection, does not affect the DDT-syndrome [42].

For the sake of completeness we mention, that according to some investigators [118, 195] the action of DDT is based, at least partially, on a direct influence on the indophenol oxydase system. This strange conclusion is drawn from a series of experiments in which all sorts of compounds, more or less related to DDT, were injected in *Periplaneta*. The compounds in question were then classified according to the degree in which the consequent symptoms coincided. Now, it appeared that among other things p-phenylenediamine and hydroquinone caused symptoms, resembling those of DDT, while death occurred after the same period of time. These compounds playing an important part in the said oxydo-reduction system, the investigators consider it likely that DDT does so too. The foundation for this assumption seems to us to be entirely insufficient, and the line of enquiry, as yet, singularly unpromising.

The main points of the literature about "DDT-metabolism" have now been dealt with. We could no more find a solution to our problem by studying metabolic processes than by a histological approach. We found some metabolic irregularities of general occurrence after DDT poisoning. However, we always noted that these were secondary effects. An exception in this respect is the theory by which DDT primarily causes hypocalcaemia. Yet we could not value this view very highly, since the data on the subject do not offer us much assistance.

We are now going to see, whether an analysis of the influence of chemical structure or physical properties of DDT may help us further.

Relation between toxicity and chemical or physical properties

Before starting our survey of the literature on this subject, we must once more bring the problem into sharp focus. We want to know where exactly in the organism DDT has its site of action. We also want to know what exactly happens there; in other words, how the DDT-molecule interferes with the chemical or physical functions of that site of action. In Chapter I we discussed a question which, although related to the chemical and physical properties of DDT, is of a different order. We do not intend to go into this latter problem, although the question of how penetration and chemical structure are related has been closely examined. The discovery of DDT as an insecticide is in fact due to such research! [94].

Surveying the large mass of facts about the toxic action of all sorts of DDT-analogues we arrive at a disheartening conclusion: The examination of almost every compound has not so much brought a solution of the DDT-problem nearer, as added a new problem of its own.

Before considering the very few valuable contributions to our problem we shall view it from a different angle.

These last-mentioned investigations have not been altogether useless. They always give an answer, though of varying clearness, to another question. This question, which is certainly of great practical value to us in our quest for new insecticides, reads as follows: "What chemical properties (e.g. structure) are essential to the toxic action?" So the interest is not directed towards that "essence" itself. From numerous investigations, in which scores of DDT-isomeres and DDT-analogues were tested, it has been possible to establish a few points of contact. We need only mention the view according to which the para-substituted benzene nucleus is of primary importance for the toxic action, this not being the case with the $-CCl_3$ group. This view, however, is also contested, but we shall not enter this field of research, as not being essential to our purpose. We shall revert to it in another publication. For literature on the subject cf. [20, 22, 23, 52, 93, 94, 107, 116, 118, 133, 160, 168, 170, 195].

If we now ask in how far the existing literature helps towards the solution of our own problem, it appears that we are still far from our goal.

It has been argued that DDT is toxic because of the liberation of hydrochloric acid at its site of action [110]. This argument is based on the fact that DDT *in vitro* is easily converted into the olefine, liberating one molecule of hydrochloric acid.

Further it appeared from experiments with a series of DDT-analogues that the capacity of a substance to split off hydrochloric acid always went side by side with toxicity, whereas analogues compounds which could not do so were not toxic.

Finally there exist other insecticides, which are chemically very different, but which also consist of C, H, and Cl, and which can also split off hydrochloric acid upon hydrolysis. Their action, moreover, resembles more or less that of DDT and the LD50 is of the same order. As an example we mention gamma-hexachlorocyclohexane and "Velsicol 1068" (γ-$C_6H_6Cl_6$ and $C_{10}H_8Cl_8$ respectively).

If the liberation of hydrochloric acid could explain the toxic action, this theory might also be applicable to these insecticides.

There are however serious objections. We ourselves performed some tests which render the theory in this form untenable. We were guided by the following consideration: if the liberation of hydrochloric acid is responsible for the toxicity, this acid itself should at least be as toxic as the insecticide producing it. It can be calculated how much hydrochloric acid can be maximally split off by the average lethal dose (LD50) of DDT, "Velsicol 1068", "Gammexane", (γ-hexachlorocyclohexane), etc.

This quantity of hydrochloric acid we injected in cockroaches without producing the least toxic effect. Even after injecting two or three times this quantity no harmful action could be observed.

It may be objected, that DDT may be concentrated through an adsorption in its site of action (e.g. in membranes of the nervous system; see below), so that a much higher HCl-concentration may occur there than following injection. But it is not likely that the liberation of hydrochloric acid actually takes place there. We have seen that it is fairly certain that the formation of DDA, at least in mammals, takes place in the liver (p. 43), while the olefine was never found in the blood.

But even if we knew nothing of all this — as is actually the case in insects —, it would yet remain inconceivable how hydrochloric acid could excercise an important and prolonged influence on the

membranes. Both Cl^- and H^+ are present in great quantities in all tissues. Besides they can easily pass all living membranes. Any accumulation caused by mass-hydrolysis of DDT would therefore immediately be neutralized by the ions in the surrounding tissue-liquids.

Other investigators noticed that the hypothesis according to which there is a relation between toxicity and the capacity of the compound to split off hydrochloric acid *in vitro*, does not hold in a large series of DDT-analogues [21, 22, 125].

It is still conceivable that the hydrolysis is of essential importance, not owing to the eliberated hydrochloric acid, but owing to the rest of the DDT-molecule. This rest is, as far as we know, nothing else but the olefine (or perhaps DDA). Neither of these compounds, however, is toxic. We see that also this last attempt to a reconciliation with the "HCl-theory" remains fruitless.

According to another view DDT intervenes somewhere in the indophenol oxydase system. We have seen already (p. 55), that there are no good grounds on which to defend this view.

More attractive is the view according to which the toxic action of DDT depends on adsorption in certain surface membranes. Especially those of the nervous system, e.g. myelin sheaths of the peripheral nerves, are brought into consideration. We are of opinion that also synapses, myoneural junctions and possibly still other structures can play a part in this respect.

This view is supported by sound experimental evidence. From experiments that will be treated later it appeared that DDA and the para-hydroxy-analogue of DDT do not act on the peripheral nerves of Arthropodes, whereas DDT, the methyl-ester of DDA and the para-methoxy-analogue of DDT do. The first two are water-soluble, the latter three are lipoid-soluble [180]. Also the action of some other compounds, such as "Velsicol 1068" and "Gammexane", which are chemically entirely different, but which have in common that they are lipoid-soluble, points to a physical rather than a chemical cause of their toxicity [cf. also 181]. We shall mention a few other arguments later.

Finally we mention a recent investigation from Sweden, in which a relation between the dipole-moment and toxicity was found [113, 114]. It appears that the insecticidal (or more generally: the toxic) properties are linked with a dipole-moment of the molecule of about

4 Debyes. Molecules with a greater or smaller dipole-moment have as a rule little or no toxic effect.

This fact is as yet not understood and moreover does not unconditionally hold for DDT. For here it is not the dipole-moment of the entire molecule that should be about 4 D., but only that of the group "R", when the molecule is written $\begin{matrix}R\\R\end{matrix}>CH—CCl_3$. There are also whole series of compounds for which this correlation is different. Further research in this direction might prove of importance.

Whether DDT interferes at some point or other with the cholinergic or the adrenergic system, and more specially whether it has an anti-cholinesterase activity, is a question that has frequently been examined and which we shall discuss later.

We must again admit that our yield has been small. Yet the "adsorption-theory" has shed some light on the action of DDT.

Influence of DDT on living matter in general

The question whether DDT has a general toxic action on the protoplasm has not been extensively studied. Yet a sufficient number of facts are known to give a well-founded answer to the question; this answer is in the negative. As far as we know only one extensive investigation into the influence of DDT on tissue cultures has been made [97]. In these experiments DDT was, in a great variety of manners, brought into hanging drop-cultures of embryonic tissues (heart and intestine of chick-embryos; brain and spleen of rats). In no case was there any sign of a detrimental influence, not even when highly concentrated emulsions (one per cent) were applied. The tissues continued to grow undisturbedly for weeks.

The authors themselves were astonished at this result, but we believe that there is nothing strange in it, because poisons can act in various ways.

We can conceive of them as divided into two groups, first, poisons fatal to protoplasm and secondly, poisons that act violently on some specifically sensitive cells, tissues and organs. When the latter occupy a key-position in the organism as a whole, the action on such a part will be fatal to the whole. So DDT belongs to the second group.

Examples of poisons that could be grouped under the first heading are HCN, $HgNO_3$, Radium, etc. Most poisons come under the second heading. Whether a

certain poison comes under the first or under the second heading often depends on the dose in which it is administered.

In mammals experiments showed that wounds of the integument heal well in spite of close contact with a fair amount of DDT powder [26, 27].

Tests with *Drosophila virilis* showed that imaginal disks of DDT-treated pupae, e.g. the rudiments of eye, leg and antennae, developed normally when they were transplanted into normal animals [8, 18]. The embryonic development of *Popillia japonica* is not affected by DDT, or at most little retarded. It was very seldom that abnormal imagines developed from DDT-treated pupae (contact action) [105, 106].

Amoeba proteus is not susceptible to DDT-suspensions of 1 ppm. Only at concentrations of 10 to 100 ppm. do the animals die, but only after 2 to 9 days. All sorts of properties and functions of the animal's protoplasm appear to be fairly unaffected by DDT [158; cf. also 89].

To conclude we mention, that, generally speaking, DDT does not affect the growth and metabolism of a number of micro-organisms, among which *Escherichia coli*, *Staphylococcus albus*, *Corynebacterium creatinovorus*, *Torula cremoris* [73].

It is certain that, apart from some exceptions, DDT as a contact-poison does not affect higher plants; further data about its possible action on plants are unknown to us.

All these negative results give us one mainstay: It is very likely, that the specific action of DDT is based on the affection of one or more *correlating* processes. We think especially of an action on the nervous system.

Chapter IV

INVESTIGATION INTO THE CAUSES OF THE DDT SYMPTOMS

Approach

From our research into the specific contact action of DDT (Chapter I) we come to the following working-hypothesis:

The toxic action in the bodies of all animals examined depends on the same principles, as the LD50 after injection is approximately the same in all cases, while the symptoms always show great similarity.

This hypothesis enables us to combine the results found in our two completely different test animals, the cockroach and the frog.

An additional advantage is that experimental and theoretical difficulties in *Periplaneta* (resulting from the animal's small dimensions and our incomplete knowledge of its normal physiology) can be avoided, by using the much more familiar frog in those cases where experiments with the cockroach are impracticable.

Every result that is found to be the same in both animals supports our hypothesis; conclusions drawn from results in one animal and confirmed by what is afterwards found in the other are so strong a support, that perhaps we may call it a proof.

It is obvious that our investigation starts from the conception that DDT acts somewhere on the nervous system. In the first place the DDT symptoms themselves suggest such an assumption, and secondly our survey of the literature has shown that there are experimental arguments for it.

It should of course be borne in mind that almost any poison may act on almost any organ. Our chief interest, however, lies in the specific action, the *first noticeable affection*, therefore, of the physiological process that can be held responsible for the symptoms. It is of importance for that reason, to work with small quantities of poison; when administering too big doses many other functions, in which we are not interested, are likely to be affected.

The time also is an important factor. We find that within certain limits the specific symptoms appear sooner if the dose is increased.

The determination of the dose required for a suitable latent period is a matter of experience.

In those cases in which we could fix the dose at will we mostly administered the LD50, sometimes two or three times as much.

Let us now consider our problem a little more closely.

It is conceivable that DDT acts on all parts of the nervous system with the same intensity, or else on some parts more intensively than on others. But it is also possible, that parts of the nervous system are resistant to DDT in the concentrations used. From which it follows that we should preferably investigate analytically the entire nervous system from receptor to effector.

This can be done in various ways. It is possible to examine only the spontaneous activity of the parts (by leading off action potentials), but it is also possible to make a further analysis of the behaviour of the parts by applying different kinds of stimulation.

There are, of course, still other aspects to the problem. Thus an investigation into the metabolism of the various parts of the nervous system could be undertaken. An investigation into the influence of DDT on the histological details of the nervous system would also be interesting. From our survey of the literature (p. 49) it can be seen among other things, that such-like investigations have indeed been undertaken. In the GENERAL DISCUSSION we shall have occasion to return to these subjects.

We have confined ourselves to the electro-physiological aspects of the problem, and to the study of the animal functions of the nervous system.

What parts of the nervous system do we have to consider for our investigation?

The nature of the symptoms observed renders it most improbable, that they are primarily connected with the autonomic nervous system. This consideration, in conjunction with the fact that the autonomic system in *Rana* offers greater experimental difficulties than the somatic system, has induced us to start our investigation with the latter. It will be seen later that this procedure was justified.

An investigation into the possible influence of DDT on the autonomic system in *Rana* would none the less be very interesting. The more so because some information concerning mammals is already available on this subject. We shall return to this later.

As a distinction between a somatic and an autonomic system in

insects from a physiological point of view is as yet disputable, it will be clear that the above-mentioned considerations do not apply here.

For our analysis it is of importance to study the action of small doses of DDT on the following parts of the somatic nervous system:

1. Receptors.
2. Peripheral nerves (both sensory and motor fibres).
3. Centres (brain and spinal cord in *Rana*, ganglia in *Periplaneta*).
4. Effectors, including myoneural junctions.

Methods

We availed ourselves of both the above-mentioned methods. For the first one, the recording of action potentials etc. (electro-encephalogram = E.E.G.), we used the apparatus of which Fig. 5 represents a simplified picture.

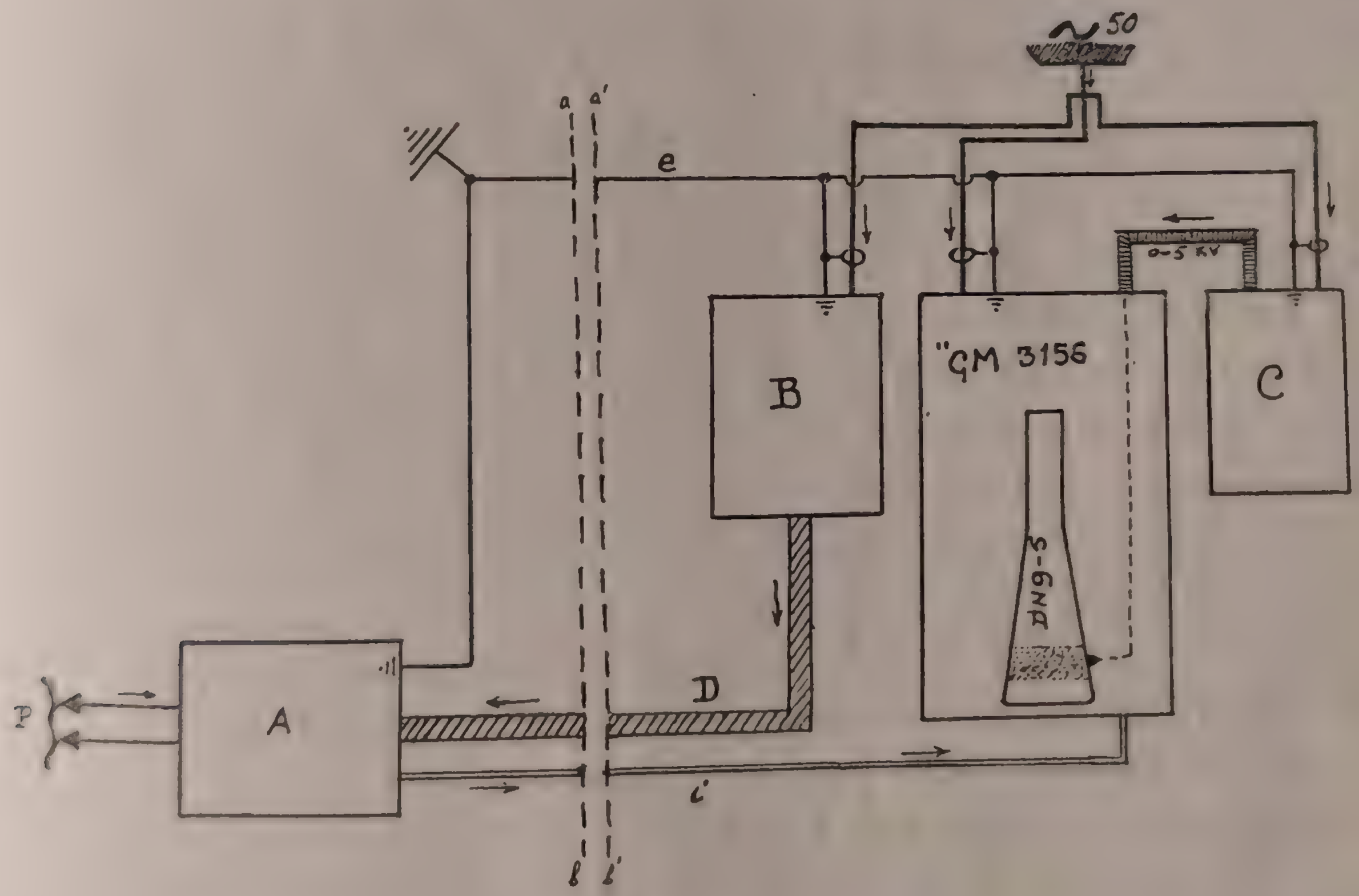

Fig. 5

Apparatus for recording action potentials etc. (simplified).

A = pre-amplifier. B = Power set supplying pre-amplifier.

C = 5 kV. power supply apparatus GM 4198. D = Leads feeding pre-amplifier.

(The leads D, i and e are shortened (ab, a′b′).)

The electric phenomena were conducted to a pre-amplifier and from there to a cathode-ray oscillograph (Philips' oscillograph type GM 3156, fitted with an intensifier tube DN 9-5 so as to facilitate photographic recording).

It would lead us too far to give an extensive description of the technical details. Such equipment has moreover been widely used in electro-physiological research [cf. 88].

It was possible to obtain undistorted recording of frequencies ranging from 5 to 1500 cycles at an amplification not exceeding 10,000,000 times. Of course we never used this top amplification, not only because the phenomenon to be recorded would thus spread far beyond the fluorescent screen, but also because the background noise would predominate the actual phenomenon. By using thin platinum wire electrodes we succeeded in reducing the background noise to a minimum, without being hampered by polarization at the electrodes.

For tests in which electrical stimulation was applied we disposed of a common induction-coil for the generation of single shocks and faradic stimulation.

Nearly all tests in which the oscillograph was used could be performed so quickly, that desiccation of the preparation was out of the question, so that mostly there was no need for a moist chamber.

Influence of DDT on receptors

There is no point in examining the possible action of DDT on all receptors, since it is especially the proprio-receptors and the tango-receptors that play an important part in the motor activity of the extremities. But we dispose of more positive arguments. When a frog with evident DDT symptoms is decapitated, the symptoms continue undisturbedly. We have but recently observed this fact. We mention the most important ones of a number of experiments in which we examined the influence of decapitation on DDT symptoms (each test with 6 animals):

1. The animals were injected with 50 γ DDT per gram b.w. When symptoms were clearly visible, they were decapitated.
2. All animals were decapitated prior to injection. Then each animal received 50 γ DDT per gram b.w.

In the animals of the first group all symptoms disappeared after decapitation. Those of the second group did not develop any symptoms at all.

These experiments might lead to the conclusion that the presence of the head is an essential condition for the development of DDT symptoms. Yet such a conclusion would be wrong! For it appeared

that the animals of the first group started producing the symptoms again, provided observation was continued for a sufficiently long time, i.e. as much as one or two hours following decapitation.

That the symptoms disappeared immediately after decapitation, was therefore a result of "shock".

The tests in which the animals were decapitated before injection are equally deceptive. We hold the enormous loss of blood caused by the operation responsible for the result, since the normal circulation, which is necessary for an effective distribution of the poison to all important organs, was thoroughly disturbed. When we avoid this difficulty by destroying only the brains (by means of a probe inserted into the *foramen occipitale*) it appears that here too, DDT symptoms occur. Something similar was found by others, as we discovered later [175, 179].

So it is pretty sure that none of the sense-organs in the head can appreciably contribute to the development of the symptoms.

Also decapitated DDT-cockroaches show distinct DDT symptoms. And furthermore we may mention, that others too have noticed that decapitation has no influence on the continuance of DDT symptoms in *Periplaneta* [171].

Experiments with Rana

If DDT is assumed to have a specific action on the proprio-receptors and tango-receptors, it may be taken for granted that that action will also manifest itself in such organs of the foot of the hind-leg. We believe, therefore, that the preparation represented in fig. 6, may serve as a prototype for our test-object. Such a preparation remains in good condition for hours and is very easy to obtain.

It should be remarked that it does not appear from the figure that the preparation is still connected with the rest of the body. This was mostly the case, however. The sciatic nerve was exposed in the upper leg and cut; action potentials were led off from the peripheral stump.

When in this preparation the IIIrd toe is bent, clear action potentials can be recorded from the sciatic nerve. Also the skin-area, when adequately stimulated (gentle touching), emits a volley of afferent impulses, which can be led off from the nerve. So long as the preparation is not stimulated, it shows a background of slight spontaneous activity: only a few occasional action potentials can be recorded from the nerve.

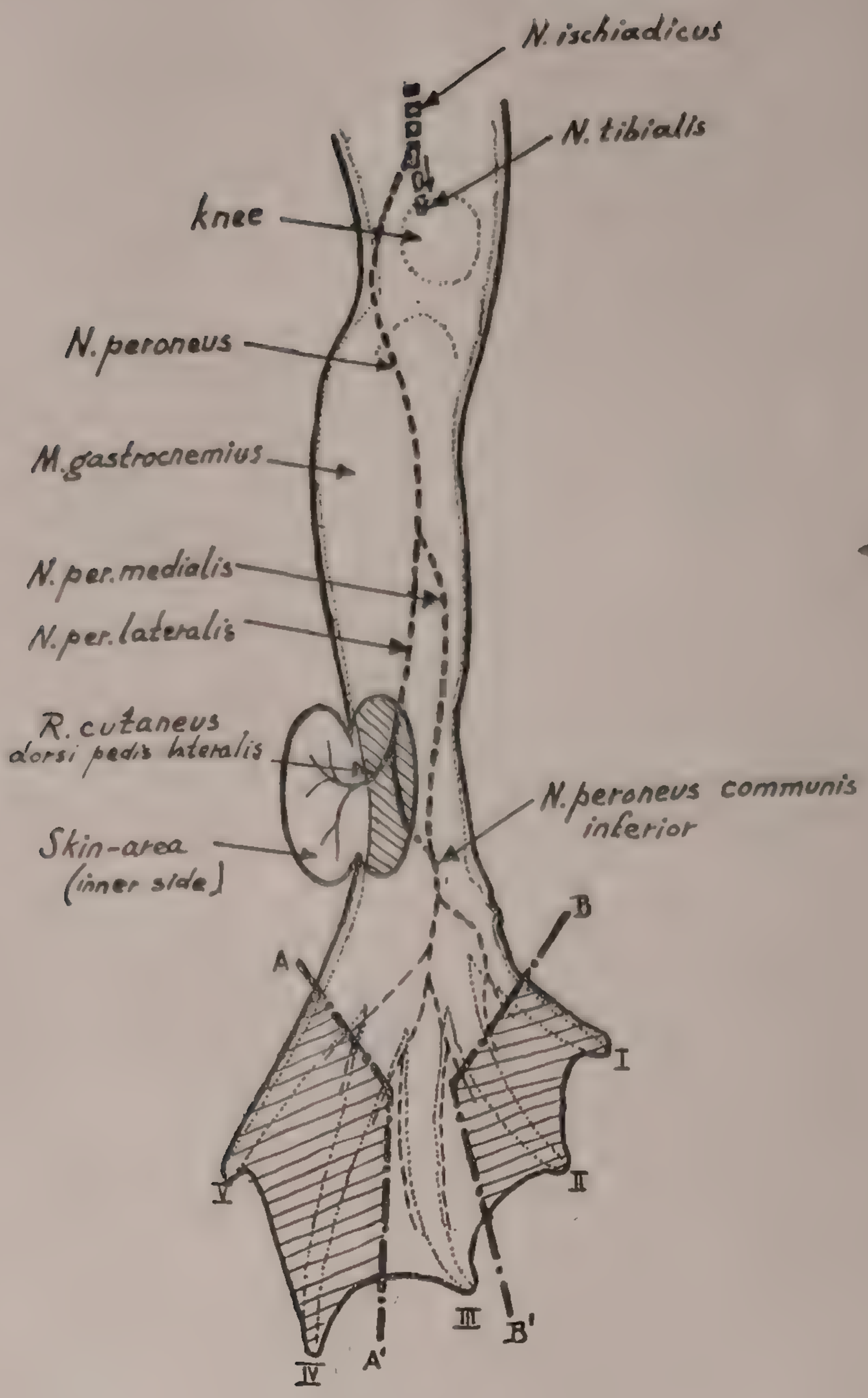

Fig. 6

Rana esculenta, preparation of a hind-leg (see text). The IVth and Vth toe have been removed by transsection along AA′; the Ist and IInd toe by transsection along BB′.

The possible influence of DDT on this preparation was examined as follows:

a. Application on the skin-area. In a large series of tests DDT was brought onto the skin-area in various forms and action potentials were led off from the sciatic nerve.

This skin-area is innervated by the *r. cutaneus dorsi pedis lateralis* (a branch of the *N. peroneus lateralis*). In a few tests the *N. peroneus communis inferior* was cut, so that we were sure not to encounter action potentials from the toe. It appeared, however, that this operation was superfluous and in most tests, therefore, the nerve was left intact (fig. 6).

By means of such a preparation we tried to discover the influence of DDT-crystals, solutions of DDT in acetone or oleum arachidis, and DDT-emulsions. (The latter three in varying concentrations.)

In none of the four cases was the activity different from that recorded from a preparation in rest. Only immediately after application of the acetone solutions did we see a temporary increase of the background-activity, but this was also seen when only acetone was applied to the skin. DDT, therefore, applied in various ways, has no stimulating effect on the tango-receptors and does not lower their thresholds in such a degree as to evoke spontaneous firing.

No more does it inhibit or paralyse these receptors as, after each test, reactions to adequate stimuli remained possible. As far as we could see, these reactions were also quantitatively identical with those of the untreated controls. From which it follows that even a slight lowering of the threshold of the sense-organs is practically excluded.

b. Application to the proprio-receptors. This presents almost insuperable technical difficulties. We therefore injected the animals before the test, mostly with 50 γ DDT per gram b.w.

When the symptoms were clearly visible (second or third stage, p. 45) the preparation was undertaken. To that end we mostly cut the *r. cut. dorsi pedis lateralis;* sometimes the skin was entirely removed.

While adequate stimulation (bending of the toe) always gave rise to clear action potentials, the general picture also remained completely normal, so long as no external stimulation was given. Here, just as in the case mentioned under *a*, we did not find any quantitative difference between the DDT-preparations and the controls, from which it appears once more that the threshold is not appreciably decreased.

With regard to the possible action of DDT on sense-organs we must therefore conclude:

DDT has no stimulating, nor an inhibiting influence on the proprio-receptors and tango-receptors in Rana.

Experiments with Periplaneta

Technical difficulties prevented us here from making a similar preparation as that which we used in the case of *Rana*. We must content ourselves with a preparation in which the *N. cruralis* (i.e. the 5th nerve, Pringle [134]) of the second thoracic ganglion was severed from it, while the distal end remained connected with the second leg (fig. 7). It is known that this nerve contains both motor

fibres and sensory fibres. The latter innervate the campaniform sensilla (proprio-receptors) and the sensilla of the hairs or spines of the leg.

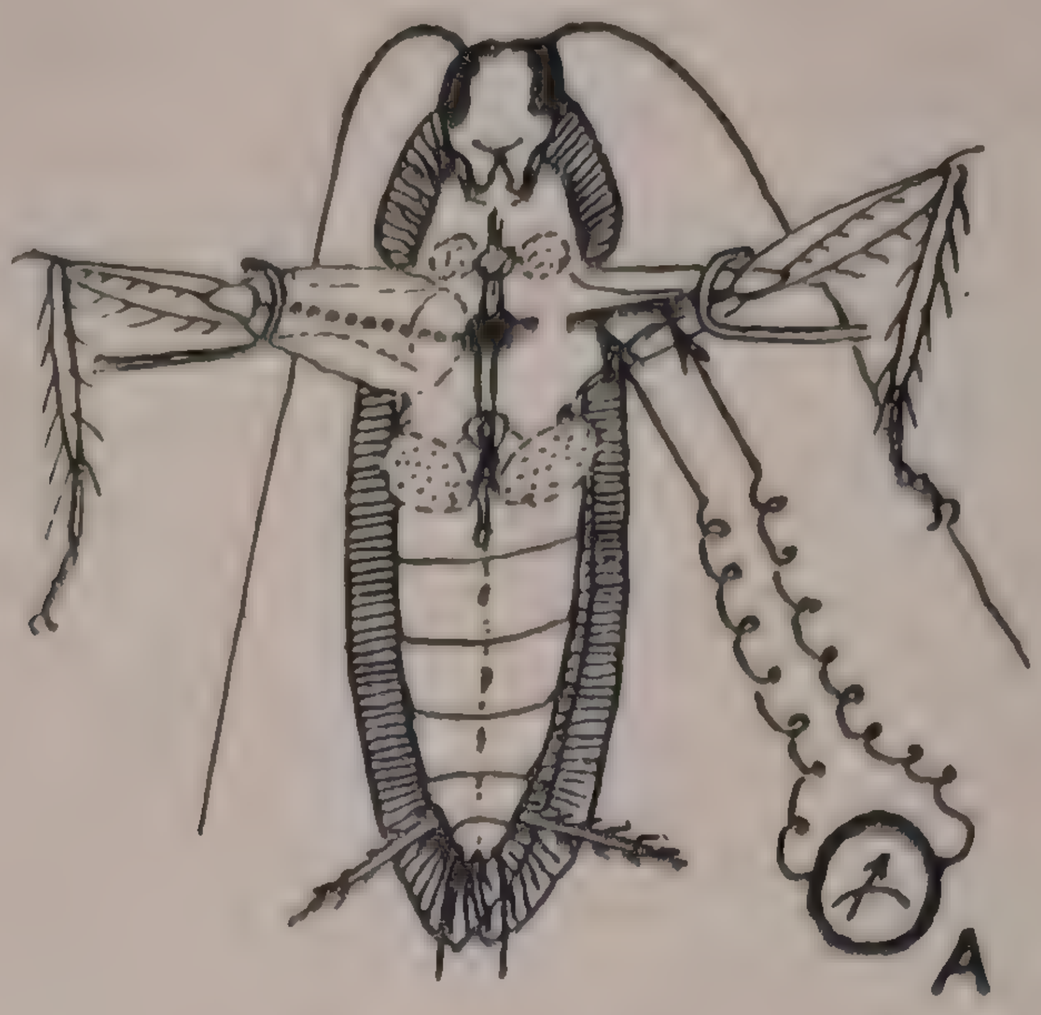

Fig. 7

Periplaneta americana

Preparation used for leading off action potentials from the peripheral part of the N. *cruralis*. The left nerve has been exposed (on the right side of the diagram). *A* represents the entire technical apparatus (cf. fig. 5).

Recording electrodes were placed on the nerve. Therefore, adequate stimulation of this preparation was bending the leg or touching the hairs and spines. In the untreated controls a background of low and medium action potentials from the sense-organs in the leg is recorded, also when they are not intentionally stimulated. It seems that very susceptible sense-organs are situated there, which respond already to weak stimuli (mechanical vibrations, sounds, etc.).

Now when the leg is bent, or when the hairs are touched, the cathode-ray registers a large volley of action potentials of varying intensity and frequency (fig. 10) [1]).

Exactly the same thing was found, when the experiments were repeated with a preparation derived from an animal that had received an injection of 20 γ DDT per gram b.w. (first, second or third stage of intoxication). Before stimulation there was not the least sign of increased spontaneous activity (fig. 11 and 12). Adequate stimulation gave the same results as is shown in fig. 10. These results were

[1]) Fig. 8—31 : at the end of this paper.

invariably obtained from a great number of preparations, so that the reproducibility of the test was beyond doubt (apart from slight individual differences in the spontaneous activity, as appears e.g. from fig. 10, 11 and 12).

Our conclusion is therefore, *that DDT has no specific action (neither activating nor inhibiting) on the sense-organs in the leg of Periplaneta.*

Literature on the subject

Our results in *Periplaneta* are in contradiction with those of the American investigators Roeder and Weiant [153, 154, 155]. They concluded that DDT does have a specific, stimulating action on the proprio-receptors in the leg of this animal. In almost the same preparation as we used, they found after DDT-injection into the tibia (of which a part had been removed) a notable increase of nervous activity (up to 7 times the normal amount). DDT-tremors, according to these authors, result from an increased reflex activity, as effected by the intense bombardment of the ganglion with afferent impulses. It is rather strange that this could be confirmed neither by other investigators [103, 180] nor by us, in a similar preparation. It is true, that some authors [e.g. 171] interpreted their experimental results in agreement with this conception, but we shall see that other interpretations were also possible in those cases. This subject will be dealt with extensively in Chapter VI.

For the present we only point out that the way in which DDT was applied was not the same as in our experiments. We prefer our own method, because it is the nearest approach to the situation in the intact animal.

To our knowledge no investigation has been conducted into the action of DDT on sense-organs in Vertebrates.

Influence of DDT on peripheral nerves

We present our conclusions with some reserve, as they are not based on a complete set of experimental data. Only when we have examined all properties of the peripheral nerves in their behaviour towards DDT, should we be theoretically entitled to draw a conclusion in this respect. From a practical point of view it is yet not always necessary to cling to all theoretical requirements.

Experiments with Rana

The action potentials of the sciatic nerve of the common nerve-muscle preparation can easily pass a region of it that has been submerged in a DDT-emulsion (p. 21). Even a submersion lasting one or two hours has no influence on the propagation of impulses.

A single induction-shock in a nerve so treated always evokes one normal twitch of the gastrocnemius muscle, the threshold for the reaction not differing from that for a normal nerve. Also to faradic stimulation such a "DDT-nerve" responds quite normally. Never did such a preparation show any spontaneous activity or other abnormalities. The same result was obtained when we used the nerve-muscle preparation from a DDT-frog in the first, second or third stage of poisoning. The reactions were even normal in the fourth stage.

In the latter experiments the muscle was of course also soaked with DDT-emulsion, owing to the injection and the following transport by the blood. This was of no consequence, even though the muscle was used as an indicator for the activity of the nerve. It will be seen below that the muscle continues to behave normally, despite the injection.

According to our present knowledge it is to be expected that an important change in the physiological condition of the peripheral nerves will be reflected, among other things, in a change in their thresholds [87]. As we could nowhere discover such a change, it is very unlikely that DDT should have a specific action on the peripheral nerves of *Rana*.

There was no reason to investigate whether injections with considerably higher doses or immersion in DDT-emulsions of higher concentrations should yet produce some effect, for we were not concerned with finding out what organs in the long run are liable to be affected by DDT, but we are looking for that organ of our test animals which is most susceptible to DDT. And that is, as far as we could see, certainly not the peripheral nerve.

Experiments with Periplaneta

In collaboration with Sobels [1]) we examined the influence of DDT on the threshold of the *N. cruralis* in *Periplaneta*. Either the

[1]) Unpublished experiments by F. H. Sobels in the Laboratory of comparative Physiology, State University, Utrecht.

preparation mentioned before (p. 67, fig. 7) was used, or we left the nerve in connexion with the ganglion, but then killed it before the test by heat. In the latter case it was much easier to place the stimulating electrodes on the very short nerve (circa 2·5 mm.).

The first and third pair of legs were removed, one of the two metathoracic legs served as a control (see below), while the nerve of the other leg was brought into contact with the electrodes. Induction-shocks (the frequency of which was regulated by means of a hand-key, and faradic stimulation) were administered. The reactions of the legs were observed by the eye. In these experiments we were not concerned with the question which axons responded to the stimuli, in other words how we had to interpret the corresponding movements of the leg. For we were only interested in the question in how far DDT could affect the reactions of our preparation.

We used preparations of normal animals with and without application of DDT-emulsion on the nerve — the latter serving as controls — and finally preparations derived from DDT-animals (first, second and third stage). All experiments were repeated in at least ten animals. There was not a single case showing a distinct influence of DDT. Only occasionally did we get the impression that a slight lowering of the threshold for induction-shocks took place. The thresholds in the controls were however so inconstant, that we could not qualify the changes observed as characteristic. SOBELS proved, by applying Na-citrate (in the same way as the DDT-emulsions were applied), that the method and technique described were indeed suitable for observing a lowering of the threshold.

These tests, however, showed something more. After DDT-application to the preparation and also in preparations derived from DDT-animals, the leg began to move even without stimulation. There appeared spasms and slower contractions. Both these contraction-types, of which the latter was most frequently observed, are in agreement with the pattern of innervation of the leg that we owe to the work of PRINGLE [134]. A similar behaviour of an "isolated" leg has also been described by others [171]. We shall return to this point, but we want to remark in this place that these movements have nothing to do with any spontaneous activity of the nerve. For if they had, it would be inexplicable why electrical stimulation did not produce an abnormal effect.

The contralateral leg served as a control in such experiments;

here also DDT symptoms occurred, but of course the influence of the electrical stimulation did not make itself felt: for, either the ganglion was dead, or the stimulated nerve was severed from the ganglion.

We never observed any spontaneous activity in the leg when the control-emulsion was applied.

Although we cannot mention any more extensive experimental data bearing directly on this point, we yet think ourselves justified in maintaining, that *there is at any rate no great influence of DDT on peripheral nerves in insects.*

Literature on the subject

Our conclusion is in general supported by the existing literature. As regards *Periplaneta* it is pretty sure now, that DDT has no specific action on the peripheral nerves. Only extremely high concentrations (equivalent to approx. 100 times the LD50) could evoke a lowering of the threshold [154, 155, 170, 172, 180]. Some investigators concluded from their experiments that there does exist a specific action here, but their conclusions are wrong [195, 196] or only very preliminary [9]. We shall raise our objections in the General Discussion.

In *Drosophila virilis* it was found, that DDT actually has a clear action on peripheral parts of the nervous system, but from these experiments it did not appear that this was the most specific action, and moreover the authors do not mention whether the peripheral nerves themselves, or the myoneural junctions are affected [8, 18, 172].

There seems indeed to exist a strong action of DDT on the motor nerves in Crustacea (cray-fish and crab). In these animals it was found that the nerves after DDT application gave many discharges after one induction shock, and even spontaneous trains of action potentials occurred. The resting potential of such nerves is lowered. It further appeared that there was an antagonism between DDT and calcium ions. According to the investigators DDT is active on these animals because it is adsorbed on the surface of the axons, where it renders the normal functions of the calcium ions impossible. We do not want to deny that these experiments were correct, but it should be remembered that these animals, as we saw on p. 28, are

very susceptible to DDT. It is therefore not impossible that the concentration at which the influence observed occurs was yet relatively very high. The quantity of DDT used was indeed rather great for these animals. It does not appear from these investigations that there is no influence of DDT on other parts of the nervous system, so that the problem has not yet been satisfactorily solved [71, 179, 180, 181, 182].

About an action of DDT on peripheral nerves in Vertebrates very little is known with certainty [163, 179]. According to TRIPOD it has no influence on motor nerves in *Rana temporaria* [175].

Influence of DDT on the central nervous system

For the present we shall only regard the question, whether DDT symptoms may result from an increased spontaneous activity of the central nervous system.

Although we understand as yet little of the cause and deeper significance of the spontaneous activity of the cell bodies, there are yet some points of connexion with our problem. The slow potential-fluctuations of the cell bodies, observed in the central nervous system of both Vertebrates (E.E.G. = electro-encephalogram) and Arthropodes [1, 13, 78, 136, 137, 138] are certainly accompanied by alterations of the thresholds of these perikarya. There are even cases known in which the normal fluctuations are so great that they periodically exceed the thresholds of the cell bodies, so that the latter emit rhythmical volleys of action potentials [see e.g. 78].

The increased activity occurring after DDT poisoning could, therefore, very well be a consequence of a strong increase of the frequency or the amplitude of such potential-fluctuations. The cell bodies would then be continually forced to produce spontaneous discharges, which are responsible for the corresponding action potentials in the nerves. It may also be, that DDT evokes such a lowering of their thresholds that *normal* potential-fluctuations in the cell bodies periodically reach the thresholds and *thus* cause the discharges. In either case such an influence of DDT must be revealed by the E.E.G.

Such correlations are well known in electro-encephalography. Certain drugs (e.g. camphor and metrazol) can produce both an increased motor activity and a modification of the E.E.G., while also

abnormalities in the E.E.G. produced by certain mental diseases in man can be connected with motor hyper-activity (e.g. the epileptic "grand mal").

The "E.E.G." of Rana

The literature on the E.E.G. of *Rana* is very limited. Practically it has only been possible to obtain a clear E.E.G. from the *lobus olfactorius* and the lateral walls of the *telencephalon* [67, 68, 98]. It further appeared possible to obtain the E.E.G. from completely isolated brains [98], which has the great advantage that there is an absolute safeguard against disturbing influences (of the heart, nerves of the head, etc.).

We succeeded in recording a reproducible E.E.G. from such isolated brains of *Rana*, which moreover agreed with the data furnished by the literature (fig. 13). In these experiments the brains were put in a small, moist chamber, where they could be preserved for hours.

For the study of the influence of DDT on this E.E.G. it is not advisable to bring the emulsion directly onto the preparation, and for two reasons. In the first place, we do not know how much DDT should be applied in this way so as to ensure that the quantity reaching the brain-tissues is similar to that which would have reached them after injection of a known dose. In the second place, we do not even know whether DDT can penetrate at all in this way. It is conceivable and even probable that the normal circulation of the blood to and in the brain is required for this. Therefore we chose a more "natural" method. When a frog after a DDT-injection showed clear symptoms, the brain was exposed and isolated and the E.E.G. was immediately recorded.

We have not examined the quantity of DDT present in the brain in this case, but that quantity is at any rate characteristic for the situation in which the symptoms develop. We have satisfied ourselves of the fact that the E.E.G. immediately after the isolation of the brain has already its characteristic feature. For when we use the preparation some hours later, the E.E.G. is still unchanged.

We made photographical records of the E.E.G. in a large series of tests. From these records, two of which are represented (fig. 14 and 15), we draw the conclusion that DDT does not affect the E.E.G.

of *Rana*. Even when before the operation the test animal was already far advanced into the 4th stage of poisoning, the E.E.G. did not show any abnormality (cf. p. 46). After what was said on p. 65 about the influence of decapitation on DDT symptoms, the reader will not be astonished by this result.

It is obvious that we should now try to discover the influence on the "spontaneous" activity of the cell bodies in the spinal cord. We have done this in a number of animals. To that end we exposed the spinal cord from the dorsal side, after decapitation. The heart was removed, as the electrocardiogram may interfere.

The "spontaneous" electrical activity of the normal spinal cord appeared to be highly dependent on the afferent impulses that are continually coming in through the peripheral nerves, also when the preparation lies completely motionless. When all peripheral nerves were cut, the activity ceased almost completely. This is in agreement with what others found in *Rana* [35].

In our opinion the test is most efficient when we examine the influence of DDT on this very slight spontaneous activity (so after transsection of all peripheral nerves).

In this way we examined the successive regions of the spinal cord in a number of animals by placing the active electrode on different places of the spinal cord, while the indifferent electrode was placed somewhere on the skin, or, better still, on the Ringer-soaked filter paper, on which the preparation was laid. The animals were previously injected with DDT-emulsion, in the way described above.

In no case did these preparations show an oscillogram differing from that seen in the untreated preparations.

Spontaneous activity of the ganglia in Periplaneta

Again owing to technical difficulties the picture we obtained of the ganglionic activity of the cockroach is less complete than we should wish it to be. We could not examine the electrical activity of the cerebral ganglion; nor were we concerned with the activity of the abdominal ganglia. But although our experiments were thus limited to the three thoracic ganglia, we believe that this limitation is permissible. For it is not likely that the thoracic ganglia, whose special function it is to serve locomotion, should respond to DDT less characteristically than the abdominal ganglia. The decapitation

experiments have already rendered it very unlikely that there should be a specific influence on the cerebral ganglion.

From a great number of animals the three thoracic ganglia together with their connectives were isolated (in insect-saline, p. 21). With one recording electrode on a ganglion and the other on filter paper the activity of the various parts of such preparations was systematically tested. Generally speaking spontaneous activity was best observed when all ganglia were still mutually linked by the connectives. There were hardly any slow potential-fluctuations; the activity was restricted to periodical outbursts of action potentials. Fig. 16, 17 and 18 illustrate by a few examples what the action of such preparations looks like.

We have also seen other pictures, but those represented here may be regarded as prototypes.

Something similar was found by others [152]. Moreover such a picture is very common for the nerve cord of other insects [see e.g. 1, 66].

Whether the electrical activity results from spontaneous discharges of the cell bodies or from other sources is irrelevant. We are only concerned with the question whether DDT exerts any influence on this electrical picture.

In preparations from DDT-animals, however, nothing particular could be observed: they were in *every respect identical with the controls*. Neither could we discover any influence in preparations that had been immersed in a DDT-emulsion during a longer or shorter period.

DDT therefore does not affect the spontaneous activity of the central nervous system of the cockroach, nor that of the frog.

Literature on the subject

Other investigators obtained practically the same results in *Periplaneta* as we did. Application of DDT-suspensions on the isolated nerve cord was ineffective [9]. Also when DDT was applied in a different way there was never any sign of an influence on the spontaneous activity of the nerve cord [150, 154, 155].

This is quite different from what was found in mammals, in which a marked increase in frequency and intensity of the electrical activity of the cerebellum and the cerebral cortex appeared. This was seen

in cats [7, 11, 36] and also in other mammals, e.g. rabbit [11], dog and monkey [43, 129].

To our knowledge there are no publications on the action of DDT on the spontaneous activity of the central nervous system of lower Vertebrates.

In CHAPTER VI we shall see how the different reactions in mammals and in *Rana* may be interpreted [cf. also 25, 172].

Influence of DDT on effectors and myoneural junctions

The violent motor activity following DDT poisoning need of course not necessarily originate in the central nervous system. It is equally conceivable that the peripheral organs, i.e. the muscles themselves, or perhaps the myoneural junctions, are affected by DDT. We must therefore also turn our attention to this side of the problem.

EXPERIMENTS WITH RANA

A very simple experiment suffices to teach us that the DDT symptoms cannot possibly result from a stimulation (or a lowering of the thresholds) of the muscles or the myoneural junctions. If we inject a frog with DDT-emulsion, symptoms develop after some time, irrespective of decerebration (p. 65). But when we destroy also the spinal cord, all symptoms cease definitely. This is in contradiction with what was found by some others [179]. However, we have repeated the experiment many times and the reproducebility of the results is beyond dispute.

When we isolate the gastrocnemius muscle together with its sciatic nerve from a DDT-injected frog that had developed clear symptoms, the reactions of this classical nerve-muscle preparation to induction shocks and to faradic stimulation of varying intensities applied to the nerve appear to proceed quite normally. Also the reactions after direct stimulation of the gastrocnemius muscle remain unaffected.

The thresholds both of the muscle and of the nerve do not change (cf. p. 70). These experiments could even successfully be carried out with preparations from animals that had entered the fourth stage of poisoning many hours previously.

Also the nerve-muscle preparation of a normal animal that had

been immersed in a DDT-emulsion for a period from 15 minutes to 4 hours still reacted normally.

All the tests here mentioned were carried out with at least *ten* individuals. The results were always the same. It is obvious that the reactions of this particular nerve-muscle preparation can be considered as typical for the reactions of the entire somatic neuro-muscular system. Therefore, we may safely conclude *that in Rana DDT does not affect the skeletal muscles, nor the myoneural junctions.*

Experiments with Periplaneta

It is not easy to derive a nerve-muscle preparation from this animal that is in every respect comparable to that of *Rana*, but, curiously enough, it can be quite easily shown that DDT in these animals — contrary to its action in *Rana* — stimulates the muscle or myoneural junction. For when we remove the legs of a DDT-animal (second or third stage) they continue to show DDT symptoms, i.e. they continue for hours to make trembling and spastic movements [1]).

Also when amputated legs of normal animals are carefully injected with DDT-emulsion at the coxa, they will show DDT symptoms. Non-injected severed legs always lie motionless (cf. p. 72).

Do we have to deal here with a specific action of DDT? Is this finding an argument against our working-hypothesis?

We shall answer these questions later, when we have discussed the influence of DDT on the reflex-arc. We wish, however, to examine one aspect of the matter a little more closely.

If the specific action of DDT in insects consists of a lowering of the threshold or a stimulation of the muscle or the myoneural junctions, all isolated legs of all animals with DDT symptoms are expected to show the above mentioned effects. But experiments show that this is not the case. We refer to Table IX, which needs no further comment.

It appeared that neither *all legs of one DDT-animal*, nor the legs *of all DDT-animals* after isolation continue to show the symptoms, and this despite the fact that all legs of all animals before isolation

[1]) Other investigators also found that isolated legs (and other parts) of insects following DDT poisoning continued to move, e.g. *Lepidoptera*, *Diptera* and also *Periplaneta* [8, 18, 60, 150, 171, 190, 196].

TABLE IX

Periplaneta, isolated legs.

After injection with DDT-emulsion all legs of all animals show clear symptoms. When amputated the following percentages continued to move:

Injection with	Total number of legs	Percentage of legs moving
10 γ DDT per animal [1]	59	10
22 γ DDT per animal	197	35
30 γ DDT per animal	76	50

1) A cockroach weighs approx. 1 gram.

did show clear symptoms. Also when we injected isolated legs of normal animals with DDT-emulsion, only part of them began to move (40 to 60 per cent). Therefore, although we have met here for the first time a characteristic action of DDT, we have not yet finished our search: the actual cause of the symptoms remains a puzzle.

LITERATURE ON THE SUBJECT

There is not much information concerning the action of DDT on muscles and myoneural junctions. Some investigators report that in mammals it is ineffective in this respect [172] and others found negative results in *Rana*, just as we did [170, 175].

The action of DDT on the isolated leg of a cockroach has been described by various authors [150, 196]. It is remarkable that some results are even quantitatively almost similar to ours [171]: not all the isolated legs of DDT-animals *(Periplaneta)* moved, but only 32 to 93 per cent (at doses varying between 20 and 130 γ DDT per animal). Several investigators, therefore, assume that DDT acts on the muscles or myoneural junctions in *Periplaneta* [9, 153, 154, 155] and also in other Arthropodes [8, 18, 180]. It should be noticed, however, that the concentration of DDT required is mostly fairly high. And a clearly formulated conclusion as to the cause of the symptoms in connexion with this influence on the muscle or myoneural junctions is nowhere to be found. Moreover, it never appears from the experiments whether there is question of an action on the muscle or on the myoneural junctions.

Chapter V

NEW POINTS OF VIEW

Introduction

When we review the experimental results thus far obtained, we hardly see any ray of hope. We have subjected our object to a thorough-going analysis, but nowhere have we found anything in the line of a contribution to the solution of our problem. It looks as if, despite this minute analysis, or even because of it, something essential has escaped our attention — and, upon reflexion, this appears indeed to be the case. For the function of the nervous system is more than the sum-total of the functions of its elements. The essence of the "nervous-system" is the "system", not the "nerves".

So what has hitherto escaped our attention, should be looked for in a connexion between the parts, each of which, when taken by itself, appeared to be insusceptible to DDT.

Reflex-arc

Experiments with Periplaneta

For the sake of a clear line of thought parts of the experiments with this animal we have so far left undiscussed.

In order to investigate the influence of DDT on the nervous system more closely, we have made some observations of preparations similar to that represented by fig. 7. We used five different types, of which fig. 8 is a scheme. In type no. 1 the entire nervous system is intact, so the crural nerve is connected both with the leg and with the 2nd thoracic ganglion, which in its turn is still connected with the other parts of the nervous system. In type no. 2 the ganglion is severed from the rest of the nervous system, by cutting the connectives between the 1st and 2nd and between the 2nd and 3rd thoracic ganglion, and moreover, all nerves, except the crural nerve to one leg. Type no. 3 we know already from Chapter IV. Type no. 4 differs from type no. 1 in that the crural nerve is no longer connected with the periphery. Finally, in type no. 5 the ganglion has been completely isolated from all nerves and connectives.

It was possible to arrange the experiments in such a manner as to enable us to make successive photographical records of the action potentials of the crural nerve in the various preparations of one and the same animal. Thus combinations were possible of the types nos. 1, 2 and 5; 1, 2 and 3; or 1, 4 and 5. This has the advantage that it considerably enhances the comparability of the results.

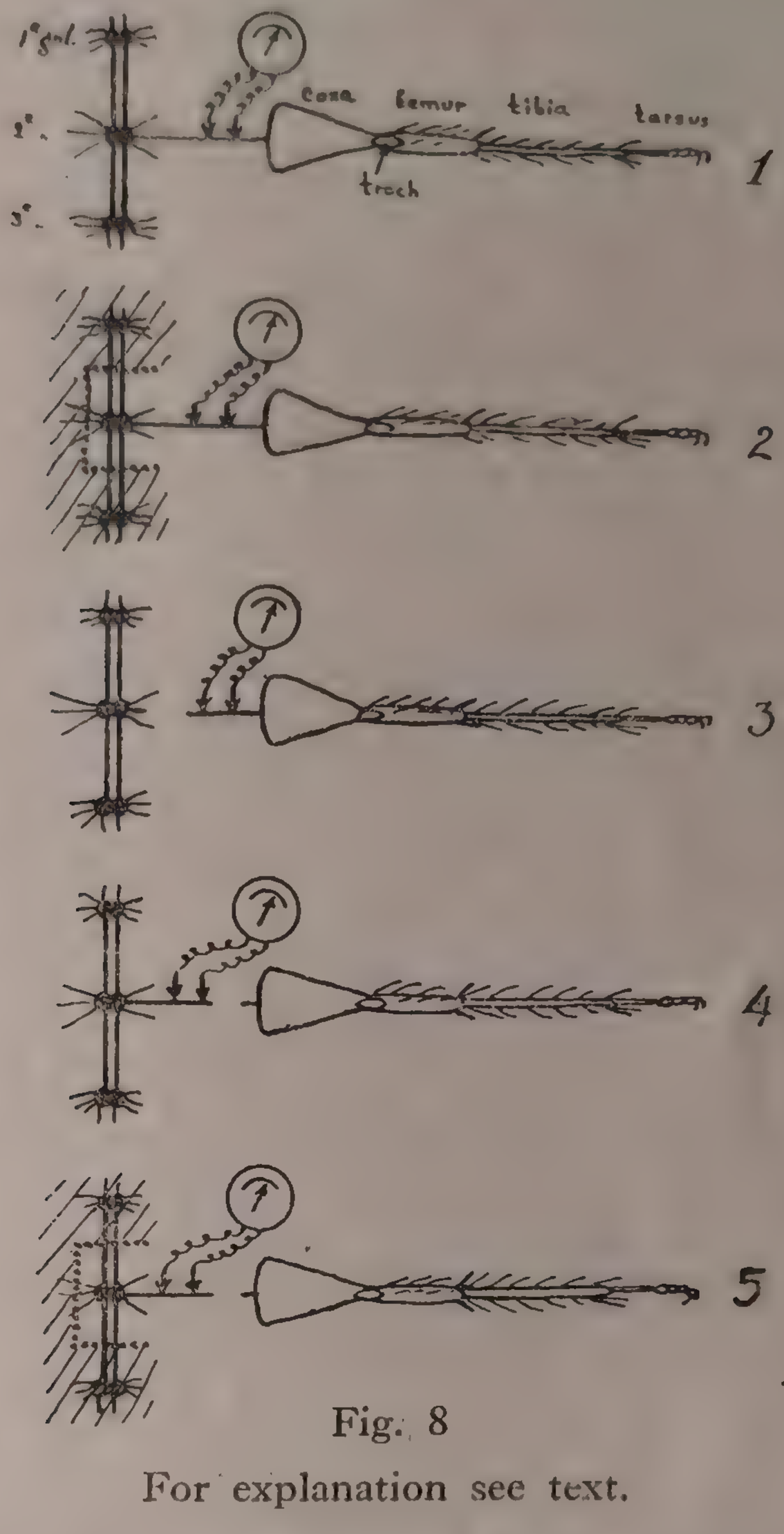

Fig. 8

For explanation see text.

These tests were repeated with a large number of animals, both treated and untreated with DDT. Of the many records we made, the few reproductions fig. 19 to 25 may serve as prototypes. We refer also to fig. 11 and 12. It should be borne in mind, that stimuli were nowhere applied; only the spontaneous activity of the preparations was recorded.

Now it appears from these records that any influence of DDT is only noticeable, *if the reflex-arc is intact*. This is most clearly shown

by the types nos. 1 and 2 (fig. 19, 20, 21 and 22) but also by type no. 4 (fig. 23 and 24), which is not so strange because here, too, afferent impulses can be set up in the crural nerve by reflex responses of the ganglia to incoming impulses from the periphery.

Type no. 3 has already been discussed in CHAPTER IV (fig. 11 and 12).

Complete isolation of the ganglion (type no. 5) results in an almost instantaneous cessation of all activity. This happens in normal animals and in DDT-animals alike: immediately upon complete isolation the ganglion emits a series of high action potentials during 4 to 12 seconds, then the activity ceases altogether. Although we have observed this invariably, it was only a few times that we succeeded in recording the phenomenon (fig. 25). Because in type no. 5 distinct action potentials were never observed — apart from this hyper-activity caused by the operation — we did not make any more photographical records.

At first sight these results are very disappointing. We did not need our complicated apparatus to discover that in DDT-animals more action potentials can be recorded from a crural nerve belonging to an intact reflex-arc, than is the case in normal animals: the DDT symptoms themselves were already a strong indication in this direction.

Yet, in combination with all our preceding observations we can gain very useful information from them. We know now that this increased activity does not originate in the periphery, nor spontaneously in the centre. In other words: in a DDT-animal *the reactions of the centre to normal series of afferent impulses are abnormally intensive*. DDT symptoms, therefore, depend on *facilitation*, i.e. a lowering of the thresholds of the *synapses*, not of the cell bodies.

The exact nature of facilitation is quite a different problem. If our conclusion should appear to be of general validity, the problem of the action of DDT would not yet be solved, but then it could at least be ranked among a mass of similar problems concerning the action of other drugs and poisons on synapses.

EXPERIMENTS WITH RANA

If the characteristic action of DDT indeed depends on a facilitation in the central nervous system, this should, according to our working-hypothesis, also be the case in *Rana*. Fortunately we know the central

nervous system of this animal so much better than that of insects, that we can without difficulty find a test which can prove the correctness of our assumption.

It is known that one single afferent impulse, which reaches the spinal cord through the dorsal root, hardly ever elicits a reflex response: the impulse is suppressed in the synapses and leaves there only a short-lived (subliminal) central excitatory state ("c.e.s." [159]). At least two stimuli of rapid succession are required to enable the synapses to transmit the impulse. In most cases still more than two stimuli are required, especially in a more or less complicated reflex-system.

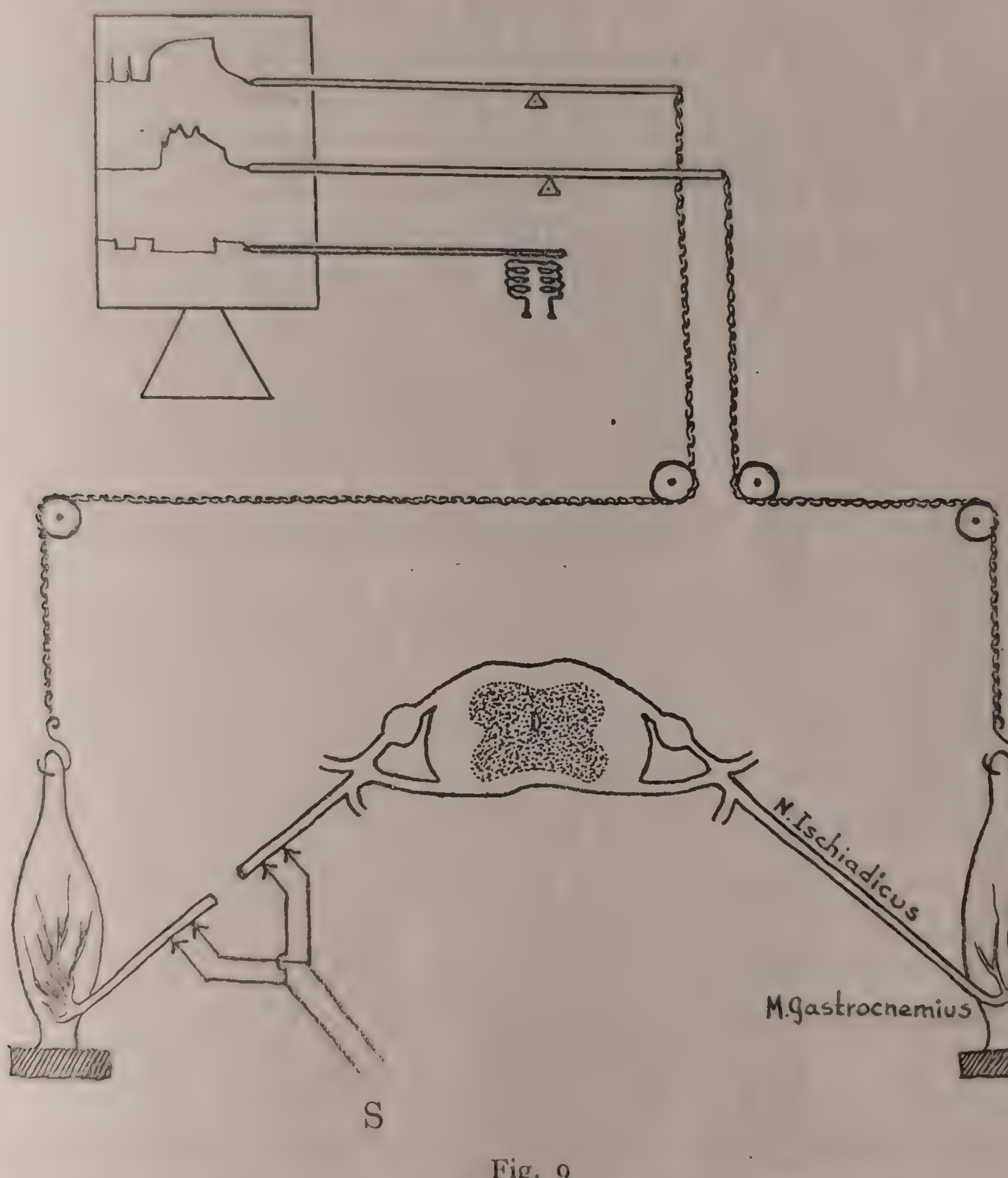

Fig. 9

Scheme of the arrangement for simultaneous registration of the homolateral and contralateral contraction. S = Stimulating electrodes.

So when we apply an afferent stimulus to the sciatic nerve of a frog (induction shock), no contralateral response will occur. In such a test we can use the contralateral gastrocnemius muscle as an indicator. The complication of the reflexes that are possible via the sciatic nerves does not make it easy to predict which contralateral response will occur after a series of stimuli. But we know for certain, that one single stimulus remains ineffective. We now arrange our test as follows (fig. 9).

The left sciatic nerve and the left and right gastrocnemius muscle of a frog are exposed. Stimulating electrodes, which are connected with a common inductorium for the generation of induction shocks or faradic stimulation are placed on the nerve. Each muscle is attached to a lever so as to obtain isotonic recording on a kymograph.

When in such a preparation the nerve is stimulated the excitatory state will be propagated to the periphery and a homolateral contraction will result. But simultaneously a signal will be sent to the centre. The stimulus enters the spinal cord through the dorsal root and can leave it through the homolateral as well as through the contralateral ventral root. So, if we want to use the homolateral muscle as a control for what happens without the participation of the centre, we must arrange the experiment a little differently. For in the present arrangement the homolateral muscle may not only receive a direct impulse through the peripheral part of the nerve, but may also receive a reflex impulse from the spinal cord.

To prevent this the homolateral ventral roots can be transsected, but such an operation is unnecessarily intricate and risky [1]). We found a much easier solution. The stimulating electrodes were so constructed that close to the nerve they branch into two pair of equal electrodes (fig. 9). The nerve was cut between those two pairs, so that the peripheral stump and the central stump could now be stimulated simultaneously and — thanks to the parallel circuiting of the electrodes — equally strongly.

The results of these experiments are shown in fig. 26, 27, 28 and 29. In preparations of normal animals the results are in agreement with what is generally known: one stimulus is ineffective, a series elicits a response (fig. 26).

When, however, the experiment is repeated with a preparation

[1]) The sciatic nerve being composed of fibres originating from a number of segments of the spinal cord, more than one root would have to be transsected.

from a DDT-animal, *it appears, in complete agreement with our hypothesis, that one stimulus, and even a very weak one, immediately elicits a contralateral reaction.* The contralateral muscle responds to every stimulus, just as if there were no spinal cord at all. The centre is "leaky", the impulses are no more retained at the synapses (fig. 27, 28 and 29).

Here indeed we have encountered the most characteristic DDT-effect, and not some accidental phenomenon. For even if we perform the experiment with a DDT-animal that has only just entered the first stage of poisoning, the result is equally clear as in an animal that has already developed violent tremors and spasms. The effect therefore does not only suffice to explain the symptoms, but is also a fundamental feature in DDT poisoning.

Whether the experiments were carried out with intact animals or decapitated ones, was of no consequence to the results. This does not surprise us, after what was said on p. 65.

It would be attractive if we could prove the existence of facilitation in cockroaches directly by means of experiments, similar to those described above. Although we are of opinion that the nature and the results of our experiments give sufficient evidence in support of our view, we thought it worth while to carry out these supplementary experiments. To our regret no results can as yet be reported as owing to various difficulties this part of our investigation is still unfinished. The greatest handicap in this respect is the limited information available concerning the electrical phenomena accompaning *normal reflexes* in the cockroach. So it is easy to put the question, but in order to answer it, a detailed investigation into the reflexes in insects must precede the proper experiments. For the time being we have to accept the fact that the grounds in this field of research are not so carefully prepared as they are in the case of Vertebrates.

As the above-mentioned experiments do not materially contribute to the solution of our problem, we have decided here to conclude our investigation. In the mean time the problem retains our attention and we hope to be able to revert to it in a following paper.

The isolated leg of Periplaneta

Before abandoning our problem we must for one moment return to our "spoil-sport": the isolated leg of a DDT-cockroach. For it is clear that the view here developed does not give a simple expla-

nation of the behaviour of such legs. We shall try to give an interpretation hereof in the light of current views and observations.

Comparative histology, anatomy and physiology of the nervous system of Evertebrates teach us that the innervation of the skeletal muscles in Arthropodes highly differs from that in Vertebrates. Many investigations have considerably deepened our insight into the significance of these differences [59, 75, 76, 108, 130, 134, 135, 188, 189, and others]. They might be summarized as follows:

1. In Vertebrates *one axon* innervates only one *small group of muscle fibres* ("motor unit"), whereas in Arthropodes *one axon* supplies the *entire muscle.*

2. In Vertebrates *one muscle fibre* receives *only one motor axon*, whereas in Arthropodes *one muscle fibre* is innervated by *two, three or even more axons*. Each of these axons serves a different type of contraction and also inhibitory axons are known.

3. In Vertebrates *each axon seldom* terminates in *more than one myoneural junction per muscle fibre*, whereas in Arthropodes such *multiple termination is the rule.*

When we translate these anatomical and histological facts into physiological terms, we see that in Arthropodes the regulation of the motor activity is largely located in the *periphery*, whereas in Vertebrates the centre serves the entire regulation. This is also reflected by the fact that in Arthropodes the centres are proportionally much smaller than those in Vertebrates.

The temptation is great to conclude from these facts that in many respects the myoneural junctions of Arthropodes actually behave as synapses. Viewed in this light it is much easier to understand how it is possible that an isolated leg of a DDT-cockroach can behave as if it contained an "excited nervous system of its own". Thus considered, DDT, in the cockroach, does not only act on the synapses but also on the myoneural junctions. So there is no need to assume that there would be an action on the muscle itself, which is in agreement with our experimental results in *Rana.*

It would not have been necessary to present this train of thought in the form of a hypothesis, if we had succeeded in blocking the myoneural junctions of a DDT-cockroach by some specific drug. For then we could have seen whether in this case the muscle itself also would cease its activity, which would be a strong argument in favour of our view.

But, although we do know a drug which specifically blocks the action of myoneural junctions in Vertebrates, viz. curare, it will be seen below that this drug turned out to be completely inactive in the cockroach. For the rest, this fact is in itself in favour of our conception! For it proves at any rate that the reaction of the myoneural junctions of the cockroach to curare differs from that in Vertebrates, while those structures have their insusceptibility to this drug in common with the synapses of these animals.

In collaboration with SOBELS [1]) we set up a small-scale investigation into the influence of curare on DDT poisoning. The tests were carried out with "tubocurarine", an active constituent of curare. When the animals were injected with 7, 15, 50, 100, or 300 γ tubocurarine per gram b.w., no noticeable effect ensued. Even the LD50 for DDT was not affected by tubocurarine (injected before, during or after administration of DDT). This investigation, however, has not yet been concluded. We hope to publish a joint report on the subject in due course.

Meanwhile it appears from the older literature that the action of curare in lower animals and Arthropodes is different from that in Vertebrates. In some animals it is known to act on the heart [46, 131], also on the central nervous system [86], but it usually does not affect the peripheral nervous system [30, 31, 142]. In the literature phenobarbital (luminal) is said to have a specific blocking action on the myoneural junctions of insects (*Drosophila*) [8]. We could not find this confirmed in *Periplaneta*. At least, we injected these animals with doses varying from 50 to 300 γ per gram without evoking even a partial paralysis.

Neither did we succeed in blocking the myoneural junctions by $MgCl_2$, despite the fact that according to some authors this salt has a curare-like action on insects [183].

In spite of all this we must as yet content ourselves with stating a hypothesis, to confirm which a special study concerning the myoneural junctions of insects is required. The first thing that should be looked for in this connexion is a specific blocking agent, while furthermore the action of certain drugs on the synapses and on the myoneural junctions should be compared.

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[1]) cf. foot-note p. 70.

Chapter VI

GENERAL DISCUSSION

We shall now try to work up our own findings and those furnished by the relevant literature into one comprehensive picture, always confining ourselves to acute poisoning. In doing so we shall consider a few questions mentioned in the literature which hitherto we have left undiscussed because they bear only indirectly on our subject and we shall see how far they fit in with our views.

In some insects it was observed that following DDT poisoning they detach one or more limbs by autotomy [60, 94, 190]. Autotomy in such animals being a normal flight reaction, we cannot see any new problem in it: it is comprehensible that animals in a state of violent agitation show such reactions.

In a few Crustacea (e.g. *Cancer* and *Cambarus*) a distinct influence of DDT on the heart was observed [179, 182]. Although there were strong indications in favour of a specific action on the ganglionic part of the heart, the myogenetic part remaining unaffected, it appears from other investigations [47] that also a neurogenetic heart (e.g. in *Stenopelmatus*) can be insusceptible to DDT. Besides, these investigations did not show that DDT acted solely on the heart, neither did they prove that the influence of DDT had anything to do with the DDT symptoms. Finally we should point out that the action observed only occurs at relatively high doses. In dogs, after injections with 75 to 100 mg. DDT per kg. b.w., the heart showed a hypersensibility to very small quantities of adrenaline [11, 129]. Others could not find any action on the heart in rabbits [27].

Therefore, although an action on the heart was indeed observed in some cases, the results are not only contradictory, but, besides, such influence is not the rule. The action on the heart has nothing to do with a specific DDT effect, so that these phenomena will not modify our views on the cause of the symptoms.

Extensive investigations have been undertaken concerning the question whether DDT intervenes in a specific way in the cholinergic system.

DDT *in vitro* was found not to affect acetylcholine, nor acetyl-β-methylcholine [173, 179]. After DDT poisoning cholinesterase activity appeared to be unchanged in all kinds of tissues in various animals, e.g. brain (rat, mouse, rabbit, monkey, frog), nerves (e.g. the sciatic nerve of a rabbit), ventral nerve cord (crayfish), salivary glands (rat), and also in blood serum (mouse, rat, monkey), etc. [19, 72, 73, 172, 173, 179]. In some cases, especially in insects, it is true that an increase in acetylcholine was found in the last stage of poisoning, but this is not astonishing in view of the violent nerve activity of the preceding stages [73, 172, 173, 179].

The experiments just mentioned prove at least that cholinesterase activity remains normal during the first stages of poisoning, so that the increase in acetylcholine is rather the result of the nervous hyper-activity than its cause.

It will be clear that the above facts do not furnish a direct contribution to our insight into the causes of the symptoms, and we may therefore drop the matter.

According to some authors DDT has a distinct action on the autonomic nervous system in mammals. This view is among other things founded on experiments concerning the influence of DDT on the blood-sugar level and on the (temporary) therapeutic action of injections with adrenaline [95], which we have mentioned in another connexion (p. 52). Also the action of DDT on the heart is by some ascribed to an influence on the autonomic nervous system (more especially on the hypothalamus) [129].

We should remark that these conclusions are as yet insufficiently founded, and that a more extensive investigation is required. On the other hand it is conceivable that also the synapses in the autonomic centres are affected by DDT. Which effect will prevail, that on the autonomic system or that on the somatic system, depends on the specific susceptibility of the two systems.

Our information concerning the influence of all kinds of drugs on DDT poisoning is rather extensive. We will mention a few examples.

Barbiturates have, according to some authors, a strong therapeutic effect on DDT poisoning in mammals [7, 36, 125, 129, 139], others maintain that the action is slight [11] or does not exist at all [25]. In *Periplaneta* a marked antagonism is said to exist between DDT and

phenobarbital [116], but we could not find this confirmed by some (preliminary) tests.

Cocaine may act therapeutically in mammals [11, 25], whereas e.g. atropine is ineffective [11, 170], in *Periplaneta* however, the latter acts as an antagonist [116]. Urethane has an antagonistic action of varying strength in mammals [11, 125, 129, 163], but it is ineffective in cockroaches [116]. We also mention that the action of strychnine, nicotine and metrazol in mammals is more violent during DDT poisoning than it is in normal animals [11, 25].

For the present we cannot judge whether these data conflict with our explanation of the DDT symptoms or not. The fact that they are often contradictory, in conjunction with the fact that in this sort of investigation often — and especially in insects — a second unknown is added to the first, compels us to venture on this field only with great prudence. We have the impression that these investigations are indeed very useful from the practical point of view of finding an antidote against DDT, but that they did not essentially contribute to the solution of the problem of the action itself.

We have reserved our criticism on some investigations mentioned in the preceding chapters until this moment, because now our arguments can be better appreciated.

We said already (p. 69) that the results of ROEDER and WEIANT are at variance with ours. The circumstance that they used a slightly different method does not sufficiently account for the contradictory results. They mention that a DDT-emulsion of 0·01 ppm injected in the leg is already effective, whereas in the intact animal symptoms do not appear at a concentration below 5 ppm.

Apart from the fact that such data are difficult to compare, we yet get the impression that something must be wrong with these experiments, for, having observed that close upon injection the liquid spreads very rapidly throughout the body, even into the distal segments of the tarsi, it is unlikely that 0·01 ppm in the leg should produce symptoms, whereas a concentration 500 times as high should be required to produce the same results in the intact animal.

We cannot, however, indicate what mistake was made.

Without checking the results of ROEDER and WEIANT others have continued this investigation with *Periplaneta*, but although interesting results were obtained, their interpretation was wrong [171].

It appeared that after DDT poisoning with *small* doses a nicotine block of the thoracic ganglion or ganglionectomy stopped the symptoms in the corresponding legs. Their conclusion, in the sense of ROEDER and WEIANT, was that afferent impulses would in this case have been the cause of the symptoms. The results are in agreement with our observations, but our interpretation is different: the ganglion is of course equally indispensible to the development of the symptoms in the case of facilitation, while it is conceivable that all symptoms stopped after elimination of the ganglion, since, as we saw before, the action on the myoneural junctions is not evident in the case of small doses (p. 79).

It appeared that when *higher* doses were administered, symptoms were also observed when the ganglion was put out of action. But in this case we do not consider the interpretation by which also the motor part of the reflex-arc is affected [171] very satisfactory (cf. p. 69).

Others, too, found that the ganglion plays a leading part in the development of the symptoms [116, 170, 172, 173], but they did not conclusively prove the existence of facilitation of synaptic transmission.

It is interesting to notice that the rapid contraction in the leg (p. 71) only remains, so long as the ganglion is intact; this is in agreement with what we found in the isolated leg. Would perhaps the myoneural junctions in *these* rapid muscles indeed resemble those in Vertebrates?

According to YEAGER and MUNSON [195, 196] DDT affects the peripheral nerves in *Periplaneta*. They conclude from certain experiments, e.g. with isolated legs, that DDT can provoke symptoms by acting on a site situated in the leg. Other experiments, in which DDT was locally applied to a thoracic ganglion led them to the conclusion that also an exclusive action on the ganglion could provoke symptoms. They also found that nicotine, by a paralysing action on the ganglion, can suppress the symptoms, but that on the other hand an isolated leg of such an animal showed symptoms again. Now they drew the surprising conclusion that DDT acts on a "site or sites common to leg and body" and they suggested "that this site or sites consists of that region of a nerve lying between the origin of its fibres in the ventral nerve cord and terminates of its fibres in the leg". This investigation itself, of which we mentioned only a few

points, commands our respect, but we cannot possibly understand the conclusions reached.

Meanwhile the reader will have noticed that the results of these authors can easily be explained by our theory.

After these considerations we can maintain our view that DDT symptoms are a result of facilitation. It has not been made clear how this facilitation itself is brought about and the investigations, mentioned above, on a possible anti-cholinesterase activity and on the influence of certain drugs do not carry us much further.

Meanwhile it is very likely that the lipoid solubility of DDT plays an important part in the mechanism of facilitation. Experiments about the action of DDT on peripheral nerves in crabs [180] showed that various DDT-analogues had a similar action as DDT, provided they were lipoid-soluble. Water-soluble analogues, such as DDA and "OH-DDT", were inactive. The action would then depend on absorption in the sheath of the axons, which is among other things supported by the fact that thin fibres are more rapidly affected than thick ones [see also 71]. We have seen already that the action on peripheral nerves only occurs at comparatively high concentrations. This is probably also the case in insects, for which some experimental evidence can be adduced [e.g. 9]. In view of the physiological and morphological analogy of the various parts of the nervous system, it seems plausible to assume that something similar happens, at lower concentrations, in the synapses (and the myoneural junctions).

Our survey is incomplete so long as we do not include a discussion of the differences found in the E.E.G. of *Rana* and of mammals.

We have seen that after DDT poisoning the E.E.G. of *Rana* remains unchanged, and that symptoms may occur independently of the brains. From various investigations it appears that in mammals (e.g. dog, cat, monkey) after DDT poisoning distinct modifications take place in the E.E.G. The greatest increase in electrical activity both in frequency and in amplitude, is seen in the cerebellum; but also in the cerebral cortex there is increased activity [7, 11, 43, 172]. These abnormal electrical manifestations resemble those following electrical stimulation of the cortex or poisoning with convulsive drugs, such as metrazol, camphor and others. There is also a correspondence with the E.E.G. of the epileptic "grand mal". It has further

been shown that these abnormalities continue, when the spinal cord is severed from the medulla oblongata [43]; and that after meso-thoracic transsection of the spinal cord the symptoms cease caudally of the section [179].

In connexion with these experiments we wish once more to point out that precisely in the cerebellum distinct histological abnormalities were sometimes found [11]. But these abnormalities are not very instructive, since they occur irregularly (p. 49); neither have investigations into the metabolism of brain tissue shed much light on these problems.

So we cannot maintain that the action of DDT in mammals is identical with that in *Rana*. Yet we do not believe that there is a difference of principle between the two cases. It should be borne in mind that the regulation of the motor functions in mammals is mainly located in the brains (especially the cerebellum), in *Rana* on the other hand, mainly in the spinal cord. So facilitation in the spinal cord in *Rana* may in principle correspond to something similar in the cerebellum in mammals. Although this point has to be further examined before a definite answer can be given, we do not find the available data to conflict with our general view.

In conclusion we want to consider if the experimental data allow of a decision whether or not DDT produces an equally strong facilitation in all synapses of the spinal cord (in frogs).

Of strychnine, for example, it is known that it affects especially the synapses between the receptor neurons and the internuncial neurons, in consequence of which an incoming impulse is spread in all directions [1]). This manifests itself in tetanic spasms, in which agonists and antagonists are simultaneously stimulated — so that the coordination of the movements is disturbed.

DDT symptoms at first sight are strongly reminiscent of strychnine poisoning, but the most characteristic difference is that in DDT poisoning coordinated movements do occur: clonic epileptiform convulsions.

The temptation is great to assume that here a specific action on the synapses of the motor neurons takes place, while those between

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[1]) It is remarkable that strychnine does not affect the synapses of *Periplaneta* [151]; it is even non-toxic for these animals [58].

the receptor- and internuncial neurons continue to function normally, and to maintain the coordination.

This assumption is supported by the fact that in those cases where histological abnormalities in the spinal cord were found, it was especially the motor areas that appeared to be affected [7, 27]. Also the experiments of TRIPOD [175], in which the action of DDT is compared with that of strychnine and phenol, may point in the same direction. Finally we point to the above mentioned fact, that strychnine (and, for example, also metrazol) acts more violently in DDT-animals than in normal ones. This is in agreement with our assumption, since now facilitation would occur in the synapses between the receptor neurons and the internuncial neurons (strychnine) as well as in those between the latter and the motor neurons (DDT) in the spinal cord.

We have raised this subject only to indicate the existing problem. For the time being, however, it is not possible to draw any definite conclusion from the scarce information available.

We shall now discuss our third problem (cf. p. 16): cause of death after DDT poisoning.

Chapter VII

CAUSE OF DEATH IN DDT POISONING

Introduction

There are few problems that are so undissolubly linked with the basic problem of Biology as is indeed the problem of Death.

Therefore we do not believe that the reader will entertain the unreasonable expectation that we shall solve the problem of the cause of death after DDT poisoning exhaustively. What concerns us here is to try to find an important physiological process, on which DDT — directly or indirectly — has not a stimulating but, on the contrary, a paralysing action.

Our task seems to be analogous to that dealing with the cause of the symptoms. Yet there is a very fundamental difference between the two. For it is *a priori* certain that the DDT symptoms are actually caused by a specific action of that compound, even though this cause may be secondary. The cause of death may however be entirely non-specific. We can, for example, very well imagine that a DDT-animal dies of the exhaustion caused by persisting spasms and tremors. We can also imagine that, for example in mammals, a high fever acts as cause of death. It is easy thus to find other causes of death (e.g. lack of food).

However, they are, all of them, not only secondary — which, *a priori*, might also have been the case with the cause of the symptoms — but in addition non-specific: for exhaustion, fever, hunger and such like are not intrinsically connected with DDT. So we must reckon with the possibility that death after DDT poisoning is always, and in all animals, caused by non-specific phenomena. In such cases however, that cause is no longer interesting from a physiological point of view. It then becomes a matter of personal choice whether one considers this or that phenomenon as the cause of death.

None the less it can also be conceived that DDT has a highly specific, paralysing action on so important a physiological process that the organism dies in consequence of this. So, if such a process

is irreversibly disturbed by DDT, we may indeed say that death is caused by this poison in a specific manner.

We now return to our test animals to ascertain with what experimental data they furnish us so as to bring us nearer to the solution of these questions.

The blocking action of DDT

Experimental results in Rana

We refer to fig. 27, 28 and 29. In the first (i.e. left) parts of these records the phenomenon of facilitation finds a clear expression. But in the following parts this seems no more to be the case. Yet the contralateral response is not normal, and finally disappears altogether. Even a great number of strong stimuli could not elicit a contralateral response.

So there must be some part in the reflex-arc that does not transmit the stimulus. Thanks to the arrangement of our experiment we can at once conclude that the peripheral nerve-muscle preparation does not play a part in this new effect, the reactions of the homolateral muscle remaining unchanged. After what we learned already about the insusceptibility of the nerve-muscle preparation to DDT this is nothing new. So we have to seek the blocked places of the reflex-arc in the centre. That the synapses claim our main attention may become clear from what will be discussed later.

From a great number of experiments the following facts appeared. After a short pause following stimulation there is sometimes a slight recovery of the original state of facilitation. According as the preparation is derived from animals in an early or more advanced stage of poisoning, the pause required is shorter or longer. If we choose for the test an animal being in the fourth stage, there is hardly any more facilitation to be seen: the central block is then complete and irreversible. Yet, even in such cases the nerve-muscle preparation still shows normal reactions.

The appearance of a central block in a preparation in an early stage of poisoning is promoted by the electrical stimulation. This appears from the fact that the central block after a short pause, in which no stimulation takes place, disappears. After renewed stimulation a central block reappears, which after rest disappears again, etc. It might be objected that the central block would have developed

anyway, as a result of a progressive poisoning during the test. But this objection is met by the fact that a preparation from an animal which is in a more advanced stage of poisoning always shows facilitation right at the beginning.

When we "explain" this phenomenon by saying that it is a result of the abnormally great exhaustion of the centre, we only shift the problem, since the phenomenon exhaustion is itself unsatisfactorily explained.

To recapitulate:

After the facilitation a central block is the first apparent effect of DDT. In an early stage of poisoning this block is promoted by the electrical stimulation and is still reversible; in later stages the block occurs spontaneously and is then irreversible.

It is very remarkable that the occurrence of the synaptic block coincides with what we called before, without any knowledge of these facts, the *last stage* of poisoning. It is also of importance that in this stage we still find a normal heart-beat, a normal E.E.G. and normal reactions of the nerve-muscle preparation.

Especially the fact that the E.E.G. is still normal in animals that seem to have died already, is very remarkable. The cell bodies themselves even in this stage are evidently not appreciably affected by DDT. This fact lends strong support to our assumption that the block occurs in the synapses. So here we have indeed found a paralysing action of DDT, which is also specific and irreversible.

We have not proved that all or most synapses are blocked, as our investigation was confined to that part of the spinal cord from which the sciatic nerve originates. But we may safely assume that the behaviour of this part towards DDT is representative for the entire spinal cord; at least for those parts which are responsible for the somatic reflexes.

The question arises whether a block of the synapses is really so serious as to cause the animal's death? With the reservation made in the beginning of this chapter, we may certainly answer this question in the affirmative. For the most essential property of an organism is its "totality", in which the several parts are by multifarious correlations synthesized into one dynamic whole.

It need not be stressed that, certainly in higher animals, the nervous system is the principle organ system bringing about these correlations.

In this respect it is noteworthy that DDT does not affect tissue-

cultures, nor Protozoa or other lower organisms. We can now better understand this, because they do not possess a nervous system.

Experimental results in Periplaneta

Thus far we have only confined our attention to the cause of death in *Rana*. How are conditions in our other test animal, the cockroach? There is a marked difference between the two as regards the duration of the second and third stage. In the frog this is a matter of a few hours, in the cockroach of some days. On the other hand the development of the first symptoms in the cockroach begins practically immediately after the injection (within 5 minutes), in the frog only after a few hours.

We do not yet know an explanation of these differences, but we can draw a vague, but yet important conclusion from them: facilitation occurs much more quickly in the cockroach than in the frog, whereas the central block develops earlier in the frog. The transition from the third to the fourth stage is much more *gradual* in the cockroach than it is in the frog.

It is therefore not unlikely that in a preparation from a cockroach that was injected, for example, one day previously, the first signs of a block will indeed be found, but this block will still be reversible.

We have checked this in some animals. To that end we made again a preparation (fig. 8, type no. 1) and recorded from the crural nerve. The results of such tests are represented by fig. 30 and 31. The large series of action potentials which we observed in the corresponding fresh preparation (fig. 20) are absent here. Instead we see periodical outbursts, relieved by periods of comparative silence.

When we assume, in accordance with the line of thought developed here, that in a number of synapses there occurs already a reversible block, while others are still in a state of facilitation, the results found are fully comprehensible. There is indeed a striking analogy with the results obtained in a preparation of a frog in an early stage of poisoning. So this is also in agreement with the fact that in the frog the first, second and third stage last much shorter than in the cockroach.

The block developing so gradually, it is not easy to ascertain the exact moment at which it becomes complete and irreversible. And the less so, as the central block, owing to the behaviour of the myoneu-

ral junctions in these animals, need not necessarily be accompanied by immobilization. For the present investigation it is sufficient to have established that DDT exerts a blocking action also in the cockroach. A better foundation of our view will not be possible until there is more information available concerning the normal physiology of the nervous system in insects.

As yet, owing to the gradualness of the process, we cannot tell with any certainty how far the block may be considered as the cause of death in the cockroach. None the less we believe that the electrical phenomena in question point to the probability that also in the cockroach the block is the cause of death.

It remains conceivable, however, that other, non-specific factors play a part. In this connexion it has often been maintained that the animals die of exhaustion [e.g.154]. We do not believe that this assumption is entirely correct. For it appeared from our tests that, when, for example, 100 animals were injected with a quantity of DDT producing a 50 per cent mortality, all animals developed clear symptoms. Now it is remarkable that the animals that survived the injection often showed symptoms for a longer period of time than that observed in those that died. This is not in agreement with the theory of exhaustion.

The isolated leg of Periplaneta

An interesting question is whether there are also indications of a block to be found in the myoneural junctions. For the time being this question does not admit of a conclusive analysis.

We have yet tried to shed some light upon it by the following test. Cockroaches were injected with a suitable dose of DDT-emulsion. After one or two days the legs were isolated and their behaviour was observed. It turned out that the movements were indeed less (in amplitude, frequency and duration) than was the case with fresh isolated DDT-legs. Moreover the percentage of legs that moved at all was smaller than in the corresponding test with fresh legs.

These results are indeed an indication of the correctness of our theory, but they are not conclusive. For the objection could be raised that in these experiments the muscles were more fatigued than they were in fresh isolated legs, that there was a deficiency of certain metabolic processes in the muscles, and so on.

We hope that we shall once have occasion to carry out a more detailed investigation into this subject. Having seen that the behaviour of the isolated leg and, therefore, probably that of the myoneural junction, is not the most characteristic feature in DDT poisoning, we are of opinion that, in order to solve our original problem, a further analysis is not strictly required.

However strange it may appear that one and the same poison causes a facilitation as well as a block, we have to accept the facts as they are. Besides it should be remarked that in pharmacology many examples of such behaviour are known, and that it is indeed the rule. We need only mention, to give a few of them, the action of various narcotics and alkaloids, such as alcohol, chloroform, nicotine and morphine, and also of acetylcholine and adrenaline.

But the exact nature of the phenomenon is not understood, although in some cases a more or less satisfactory explanation has been given.

Discussion and review of the literature

It is not mere accident that the literature only seldom contains a pronouncement upon the cause of death in DDT poisoning: so long as the cause of the symptoms remains unexplained, it is impossible to understand the cause of death, unless it should be non-specific. But we saw that a highly specific action is responsible for the death of our test animals.

It is therefore easily understood that the literature on the subject furnishes information of a rather speculative character, and that only non-specific causes are suggested.

The received opinion is that insects die owing to general exhaustion [154, 180]. We saw already that objections can be raised to this conception, whereas our view is supported by more convincing evidence. The investigations according to which DDT exerts a specific action on the peripheral nerves of the crab and cray-fish [179, 181], did not give a single clue as to a definite conclusion. We observed already (p. 73) that these experiments do not exclude the possibility of an action on the synapses in these animals. So it remains conceivable that here, too, facilitation occurs, followed by a block.

Far more difficult is the problem in mammals. When describing the symptoms (p. 46), we mentioned that these animals usually die already in the third stage. We may therefore assume that a block would occur, if only the animals would live long enough, but such a consideration is of little value. Only one conclusion can be drawn: if the animals die previous to the development of the synaptic block, it is to be expected that the cause of death is non-specific in this case.

Now it appears indeed that various authors found such non-specific causes of death. It is not surprising that many of these findings are contradictory (cf. p. 95). In consequence of violent spasms, for example, the interruption of the respiratory movements may become so serious that the animals die of asphyxia [11, 27, 171]. Others mention that death is a consequence of general exhaustion, of hepatic injuries [27], of hypoglycaemia [95] or of fever [96]. Sometimes, e.g. in dogs, a fatal reaction of the heart was observed (ventricular fibrillation) [11, 129].

So it is easy to see that these non-specific causes of death occur in mammals, because here the poison is really not given a fair chance to exert its specific blocking action in the fourth stage. We shall not blur our general picture of the cause of death by offering a further analysis of these non-specific phenomena. It suffices to our purpose to have elucidated the following points: although it appears that, generally speaking, DDT does not exert a blocking action in mammals this is not in contradiction to our general views concerning the cause of death. In mammals death occurs sooner, only as a result of non-specific phenomena, which, apparently, are less serious in other animals. Here the homoiothermia of mammals certainly plays an important part. It will be clear that respiratory insufficiency, increased body-temperature and circulatory disturbances are less serious in poikilothermic animals than they are in the homoiothermic mammals.

It would be wrong to suppose that the problem of the cause of death would now be completely solved. There remains not only the question when an animal is really dead, but besides there are a few cases which do not easily admit of our method of interpretation. We have especially in mind the susceptibility to DDT of certain Coelenterates (cf. p. 30). In these animals the part played by the nervous system as an integrative principle in the organism is probably far less important than it is in Arthropodes and Vertebrates; none the less they died after DDT treatment.

For the present we must leave these questions unsolved, as we do not dispose of sufficient experimental data.

In spite of such teasing questions the fact remains that the cause of death in Arthropodes and Vertebrates can be explained by one and the same principle, just as was the case with the cause of the symptoms. This fact in itself is an additional support for our working-hypothesis.

The action of DDT may finally be characterized as follows:

> "*DDT is a poison that penetrates the integument of Arthropodes in a specific manner, and then, just as is the case in Vertebrates after injection or oral administration, by a specific action on the synapses, causes a prolonged state of general facilitation, followed by an irreversible block of those synapses. The integrative action of the nervous system is thus rendered impossible, in consequence of which the animal, as a dynamic whole, dies.*"

An interesting consequence of our investigation was that in studying the lethal action of a poison, we were compelled to reflect upon the innermost nature of Life.

Would it indeed be impossible to understand Death before we have fathomed Life . . .?

SUMMARY

1. — This publication deals with three main problems concerning the action of DDT:

a. What is the cause of the specific action of DDT as a contact poison in insects?

b. How are DDT symptoms produced?

c. What is the cause of death in case of DDT poisoning?

Besides, it gives a comprehensive critical discussion of the literature on the influence of DDT upon physiological processes. The one restriction made is that only the consequences of *acute* poisoning are considered.

2. — The first problem (sub 1a) is examined by comparing the LD_{50} of DDT after injection in *Rana* and *Periplaneta*. In both cases it appears to be approximately 20 γ per gram animal.

3. — The literature dealing with the action of DDT as a contact poison is extensively discussed.

4. — The experiments (sub 2) and the relevant literature lead to the conclusion that the specific contact action of DDT in Arthropodes is determined by the mechanism of penetration. In this connexion the lipoid-solubility of DDT and the particular properties of the chitinous cuticle are of fundamental significance.

5. — The literature dealing with the "intermediary metabolism" of DDT is critically discussed in a separate chapter. Attention is paid among other things to the metabolite DDA, the excretion of DDT with the urine, the bile, the faeces and the milk, and the accumulation of DDT in the tissues.

6. — A short characteristic of DDT symptoms is given. In the process of progressive intoxication four successive stages are distinguished.

7. — The literature dealing with the consequences of DDT poisoning is extensively discussed. In this connexion attention is paid to the influence of DDT on histological structures, on metabolism, and on the living matter in general. Moreover, the relation between

toxicity and chemical or physical properties is discussed. In considering all these questions the main point is to find out whether they can shed some light on the problem of the cause of the symptoms. Generally speaking this appears not to be the case.

8. — As there is evidence that DDT affects some part of the nervous system, an investigation is undertaken in this direction.

9. — After a great number of experiments, in which the frog and the cockroach are compared, it is concluded that DDT has neither a stimulating nor an inhibiting action on proprio-receptors, tango-receptors, peripheral nerves, myoneural junctions and muscles.

Nor are there any signs of an influence on the spontaneous activity of the central nervous system. Only in cockroaches the compound appears to affect the myoneural junctions, although they are not the principal site of action.

10. — The conclusion is drawn that DDT is likely to have a stimulating action on a "link" between the parts separately examined and found insusceptible, viz. the *synapses*.

11. — The correctness of this conclusion is confirmed by other experiments. DDT symptoms, therefore, depend on a *facilitation* in the central nervous system.

Besides it is made probable that the action on myoneural junctions in *Periplaneta* in principle corresponds to the action on synapses.

12. — The stage of facilitation passes into one in which the synapses are *blocked*, so that incoming impulses are no longer transmitted. This results in a decrease of hyperactivity, and finally in complete immobility, so that the synaptic block can be considered as the cause of death in DDT poisoning. In this stage the reactions of the peripheral nerve-muscle preparation to electrical stimulation are still normal, while, for example, also the electroencephalogram *(Rana)* is unchanged. Mammals die before this stage is reached, in consequence of non-specific phenomena.

13. — DDT acts similarly in *Rana* and in *Periplaneta*, while it is highly probable that also the cause of death in the two animals is the same.

14. — A critical discussion on the cause of death is given, from which is obtained additional support to the view that DDT acts similarly in Vertebrates and Arthropodes.

15. — The action of this insecticide can thus be summarized: "*DDT is a poison that penetrates the integument of Arthropodes in a specific manner, and then, just as is the case in Vertebrates after injection or oral administration, by a specific action on the synapses, causes a prolonged state of general facilitation, followed by an irreversible block of those synapses. The integrative action of the nervous system is thus rendered impossible, in consequence of which the animal, as a dynamic whole, dies.*"

SAMENVATTING

1. — Deze publicatie heeft betrekking op drie grondproblemen over de werking van DDT, nl.:

a. Wat is de oorzaak van de specifieke contactwerking van DDT?

b. Hoe ontstaan de DDT-symptomen?

c. Wat is de doodsoorzaak bij een DDT-vergiftiging?

Daarnaast werd de literatuur over de invloed van DDT op de physiologische processen zo volledig mogelijk kritisch besproken. Er werd alleen deze beperking gemaakt, dat slechts de gevolgen van een *acute* vergiftiging ter sprake kwamen.

2. — De eerste vraag (sub 1a) werd onderzocht door de LD_{50} van DDT na injectie te vergelijken bij *Rana* en *Periplaneta*. In beide gevallen bleek deze ca. 20 γ per gram dier te zijn.

3. — De literatuur over de contact-insecticide werking van DDT werd uitvoerig besproken.

4. — Op grond van de proeven (sub 2) en dit literatuuroverzicht werd geconcludeerd dat de specifieke contactwerking van DDT bij Arthropoden bepaald wordt door het mechanisme van binnendringing. In dit verband zijn de lipoid-oplosbaarheid van DDT en de bijzondere eigenschappen van de chitineuze huid van fundamenteel belang.

5. — De literatuur over de „ver- en bewerking" van DDT in het lichaam werd in een apart hoofdstuk kritisch besproken. Daarbij kwamen o.m. ter sprake: het stofwisselingsproduct DDA, de excretie van DDT in de urine, de gal, de faeces en de melk, en de accumulatie van DDT in de weefsels.

6. — Een korte karakteristiek van de DDT-symptomen werd gegeven. Het proces van de voortschrijdende intoxicatie werd in de beschrijving verdeeld in 4 opeenvolgende stadia.

7. — De literatuur over de gevolgen van een DDT-vergiftiging werd uitvoerig besproken. In dit verband werd aandacht geschonken aan de invloed van DDT op histologische structuren, op het metabolisme, en op de levende substantie in het algemeen. Voorts werd

het verband tussen toxiciteit en chemische of physische eigenschappen besproken.

Bij al deze beschouwingen werd steeds nagegaan of de resultaten enig licht konden werpen op het probleem van de oorzaak der symptomen. Dit bleek in het algemeen niet zo te zijn.

8. — Daar veel er op wees, dat DDT ergens op het zenuwstelsel werkt, werd een eigen onderzoek over dit punt ondernomen.

9. — Na uitgebreide proeven, waarin steeds de kikker en de kakkerlak vergeleken werden, kon geconcludeerd worden dat DDT geen stimulerende (noch een remmende) invloed heeft op proprioreceptoren, tangoreceptoren, perifere zenuwen, motorische eindplaten en spieren. Evenmin bleek iets van een invloed op de spontane activiteit van het centrale zenuwstelsel. Alleen bij de kakkerlak bleek, dat de stof toch wél op de motorische eindplaten kan werken, al is dat niet de enige of de belangrijkste plaats van werking.

10. — De conclusie werd getrokken, dat DDT waarschijnlijk prikkelend werkt op een „schakel" tussen de afzonderlijk onderzochte en blijkbaar ongevoelige delen, nl. de synapsen in het centrale zenuwstelsel.

11. — Door een geschikte proef kon de juistheid van deze conclusie bewezen worden. De DDT-symptomen berusten dus op een *facilitatie* in het centrale zenuwstelsel. Daarnaast werd het waarschijnlijk gemaakt, dat de werking op motorische eindplaten bij *Periplaneta* in principe overeenkomt met de werking op de synapsen.

12. — Het facilitatie-stadium gaat over in een stadium waarbij de centra „verstopt" zijn, doordat nu de synapsen geblokkeerd worden. Dit verschijnsel gaat gepaard met immobiliteit, en het kan beschouwd worden als de doodsoorzaak bij de DDT-vergiftiging.

In dit stadium is de reactie van het perifere spier-zenuwpreparaat op electrische prikkeling nog normaal, terwijl bv. ook het electro-encephalogram *(Rana)* ongewijzigd is.

Zoogdieren sterven vaak vóór dit stadium bereikt wordt, ten gevolge van niet-specifieke oorzaken.

13. — DDT werkt zowel bij *Rana* als bij *Periplaneta* op overeenkomstige wijze, en ook de doodsoorzaak is in beide proefdieren vrij zeker dezelfde.

14. — De literatuur over de doodsoorzaak werd kritisch besproken. Daaruit kon nieuwe steun worden verkregen voor de opvatting, dat DDT bij Vertebraten en Arthropoden op dezelfde wijze werkt.

15. — De werking van dit insecticide kan aldus worden gekarakteriseerd:

„DDT is een vergif dat bij Arthropoden op specifieke wijze via de huid binnendringt en dan, juist als bij Vertebraten na injectie of opname per os, door een specifieke werking op de synapsen een aanhoudende toestand van facilitatie opwekt, gevolgd door een irreversibele blokkering van die synapsen. De integrerende functie van het zenuwstelsel wordt daardoor onmogelijk, ten gevolge waarvan het dier als 'dynamische eenheid' sterft."

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I.D.P. = Insecticide Development Panel

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Fig. 13

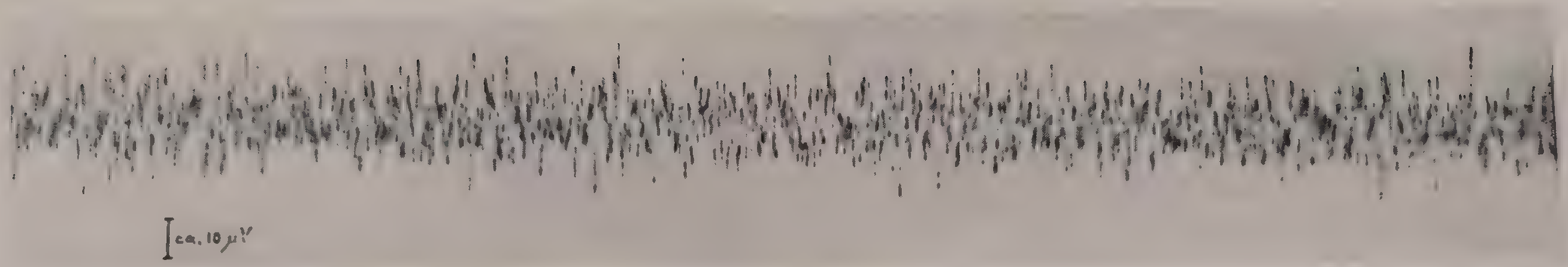


Fig. 14

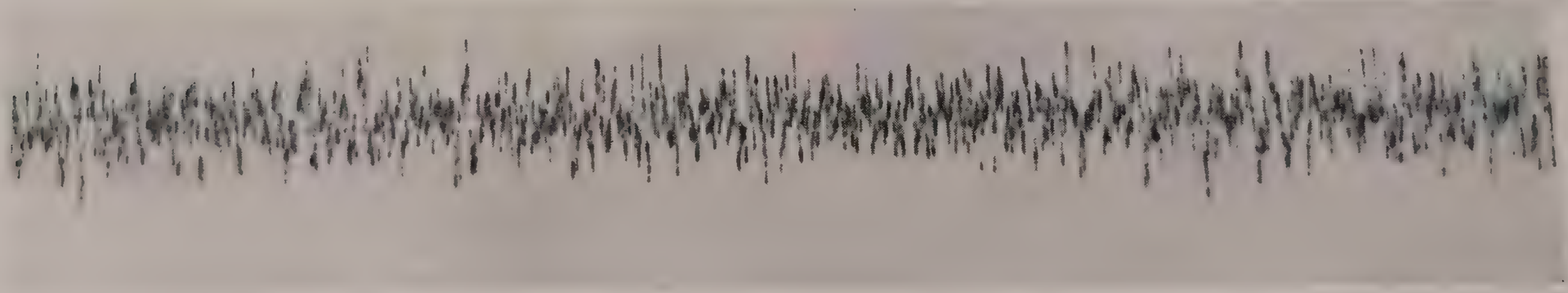
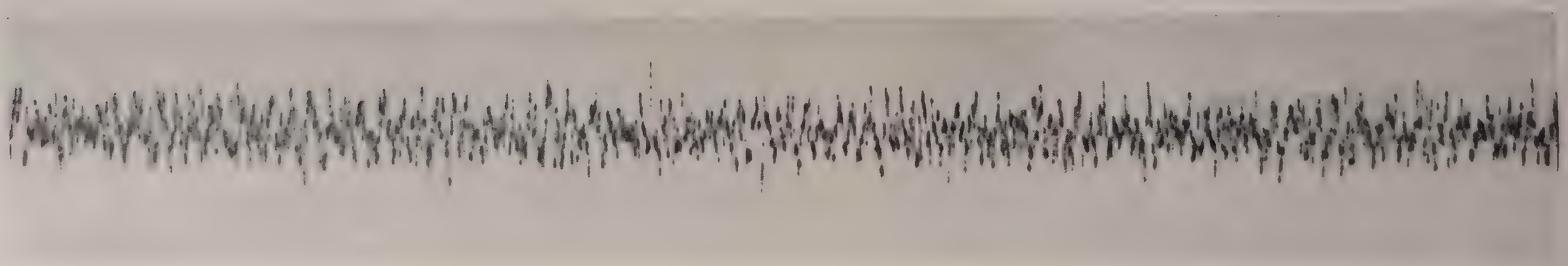


Fig. 15



Rana esculenta, electroencephalograms. Fig. 13: normal animal; Fig. 14: DDT-treated animal (2nd stage); Fig. 15: DDT-treated animal (4th stage). Amplification the same in all records. Speed of film: see time signal II. For explanation see text p. 74 (scale 3/5).

Fig. 16

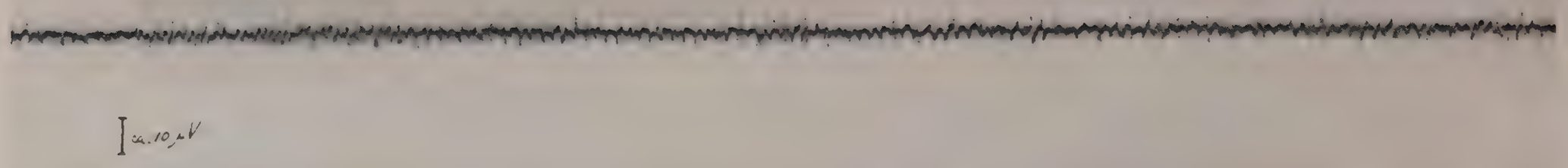


Fig. 17

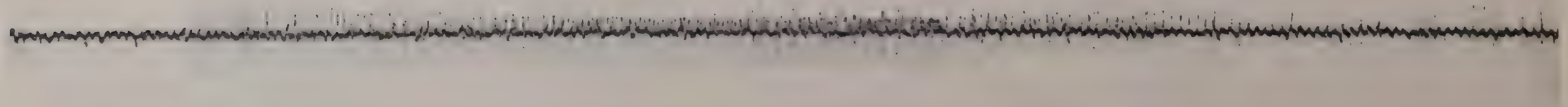
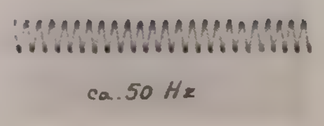


Fig. 18



III
Scale $^{1}/_{2}$

Periplaneta americana, spontaneous activity of central nervous system. Amplification the same in all records. Speed of film in Fig. 16: see time signal I; in Fig. 17 and 18: see time signal III. For explanation see text p. 76 (scale Fig. 16 $^{3}/_{5}$; Fig. 17 and 18 $^{1}/_{2}$).

Fig. 19

(type no. 1)

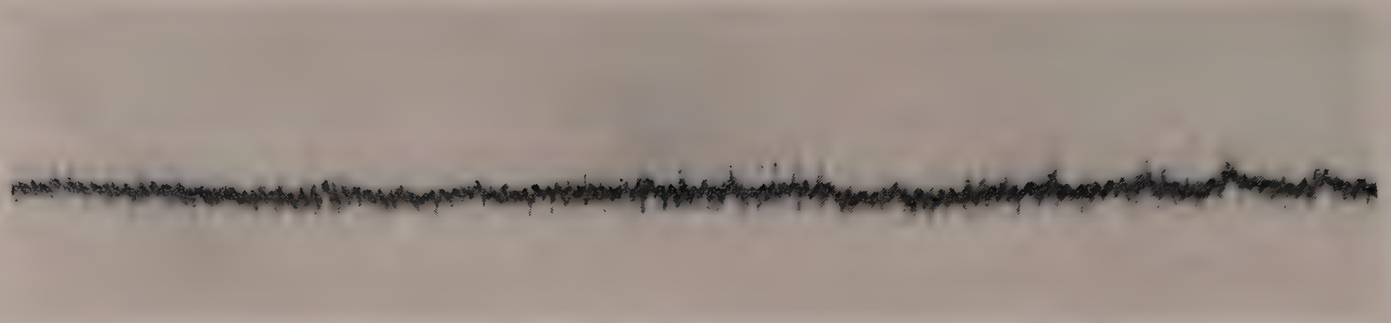


Fig. 20

(type no. 1)

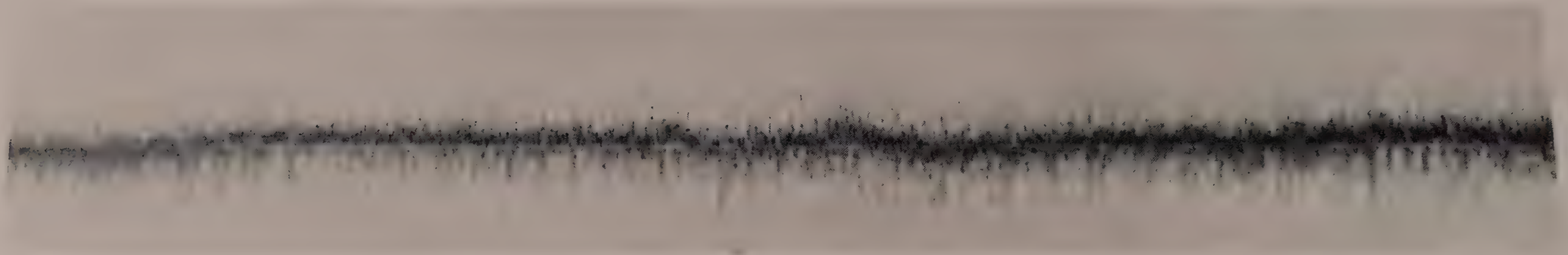
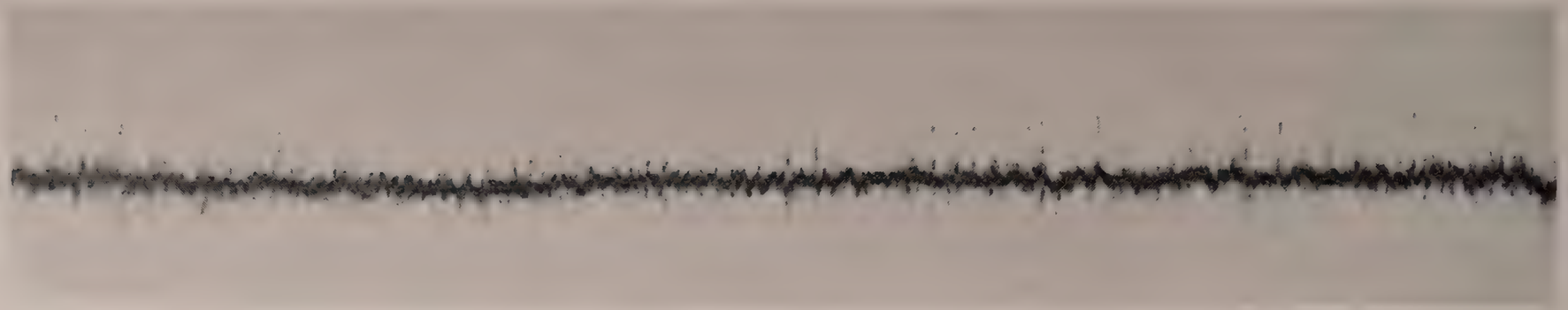


Fig. 21

(type no. 2)



Periplaneta americana, action potentials. Amplification the same as in Fig. 24. Speed of film: see time signal II. Fig. 19 and 21 normal preparations, Fig. 20 DDT-treated preparation. For explanation see text p. 81 (scale $^{3}/_{5}$).

Fig. 22

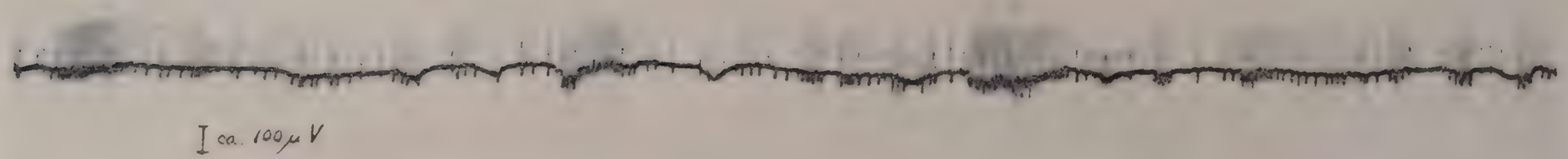
(type no. 2)

Fig. 23

(type no. 4)

Fig. 24

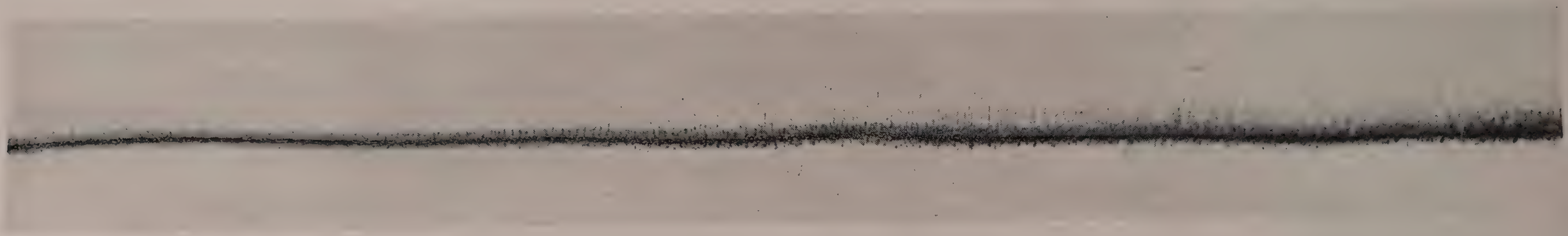
(type no. 4)



Periplaneta americana, action potentials. Amplification the same in all records. Speed of film: see time signal II. Fig. 22 and 24 DDT-treated preparations; Fig. 23 normal preparation. For explanation see text p. 81 (scale $^{3}/_{5}$).

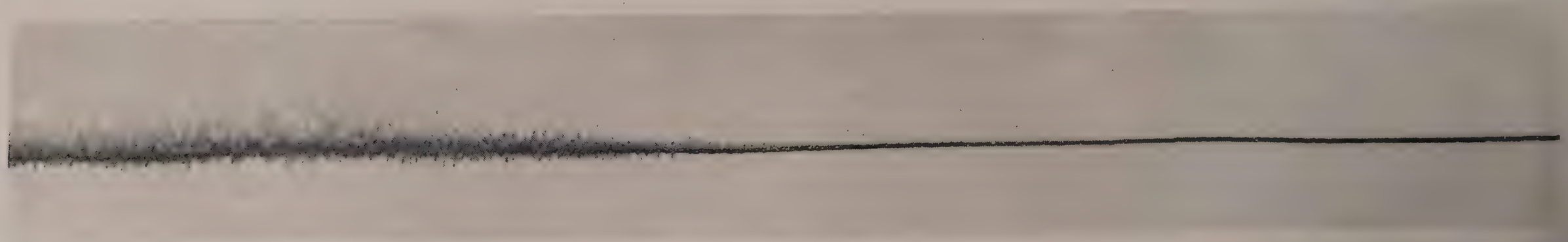
Fig. 25
(type no. 5)

1st Part



2nd Part

*



Periplaneta americana, action potentials. At * the record is cut into two parts. Amplification the same as in Fig. 24. Speed of film indicated by time signal II. For explanation see text p. 82 (scale $^1/_2$).

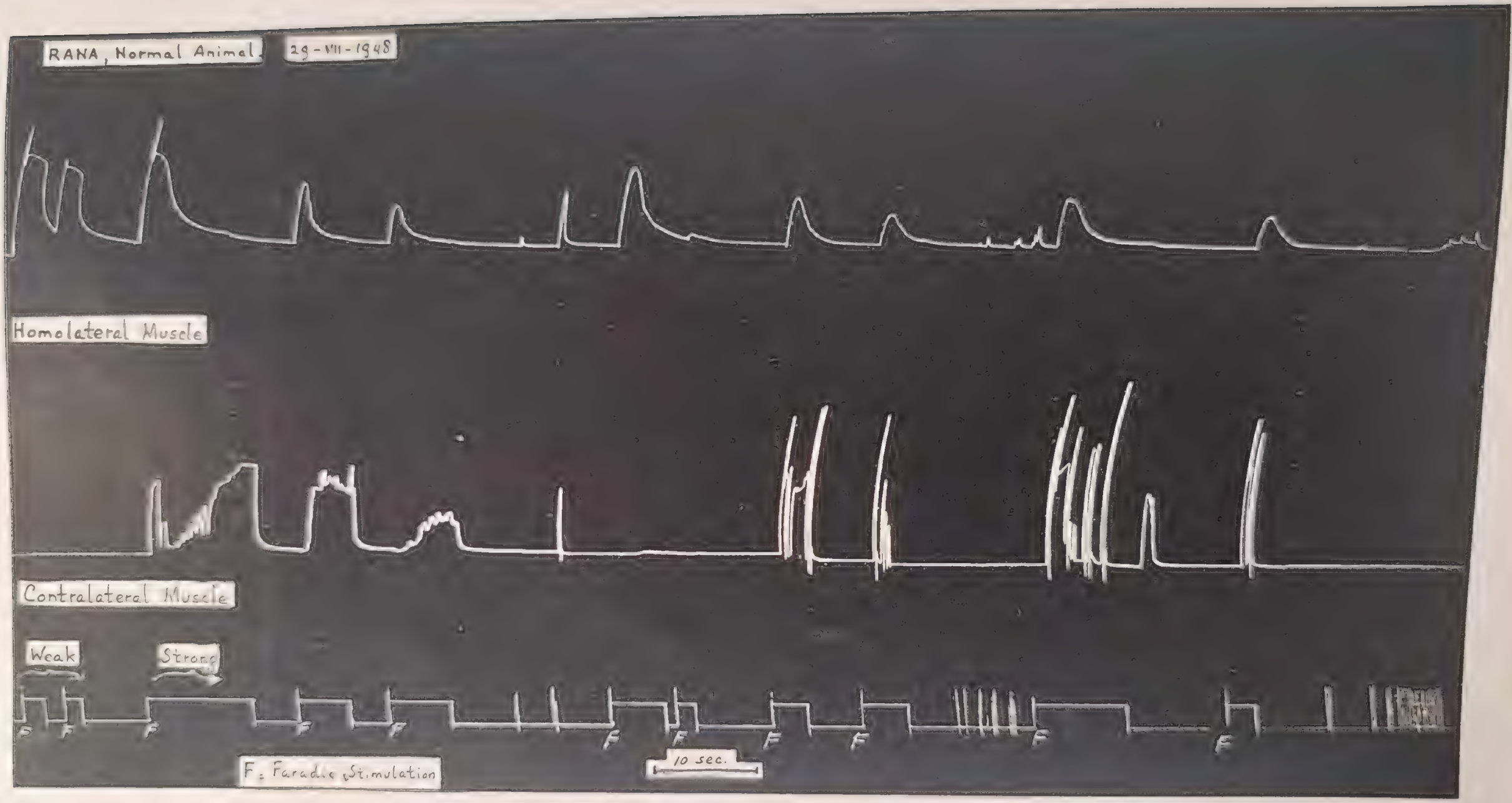


Fig. 26

Rana esculenta, mechanograms of the gastrocnemius muscles. For explanation see text p. 84.

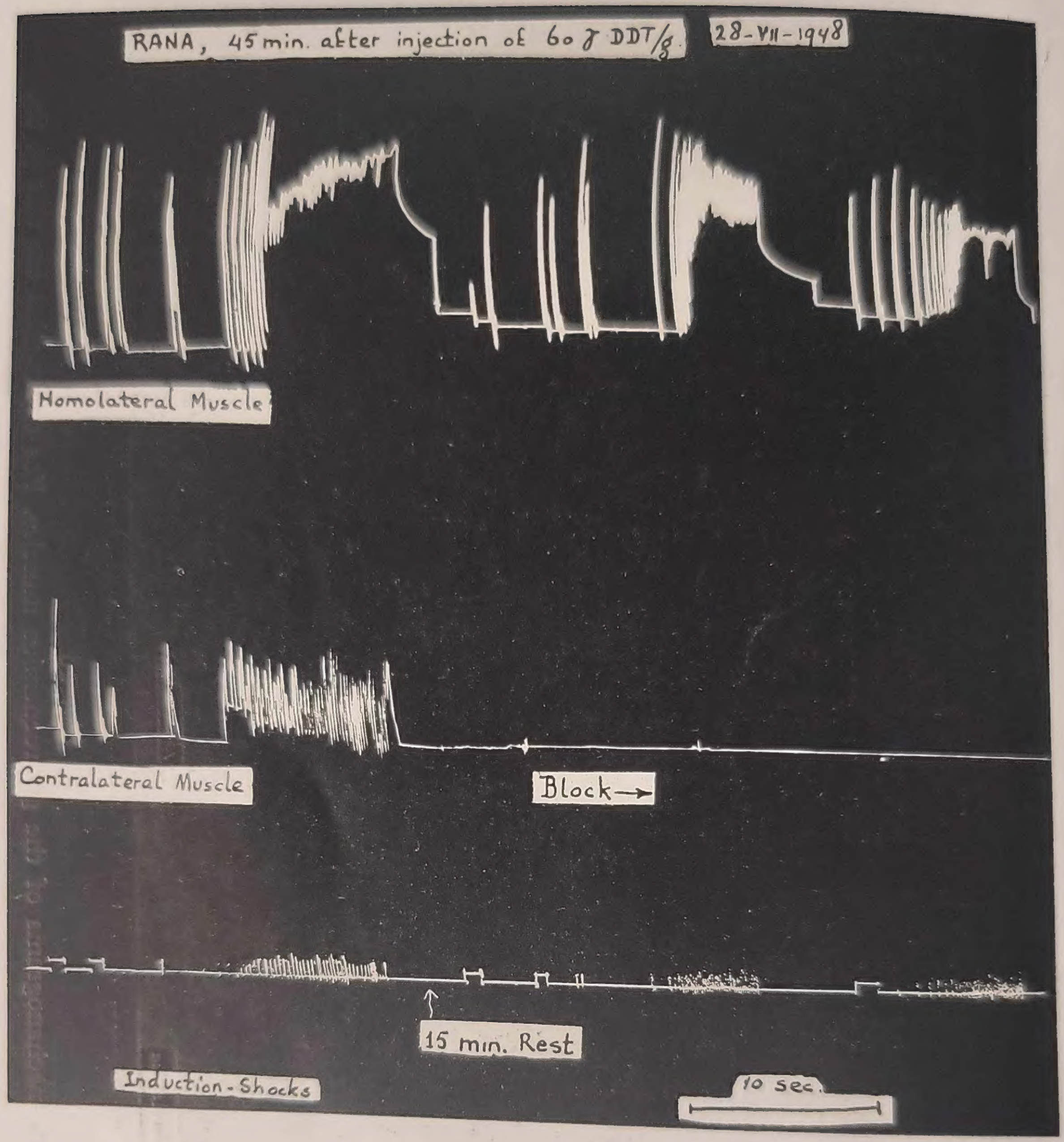


Fig. 27

Rana esculenta, mechanograms of the gastrocnemius muscles. For explanation see text p. 85 and 96.

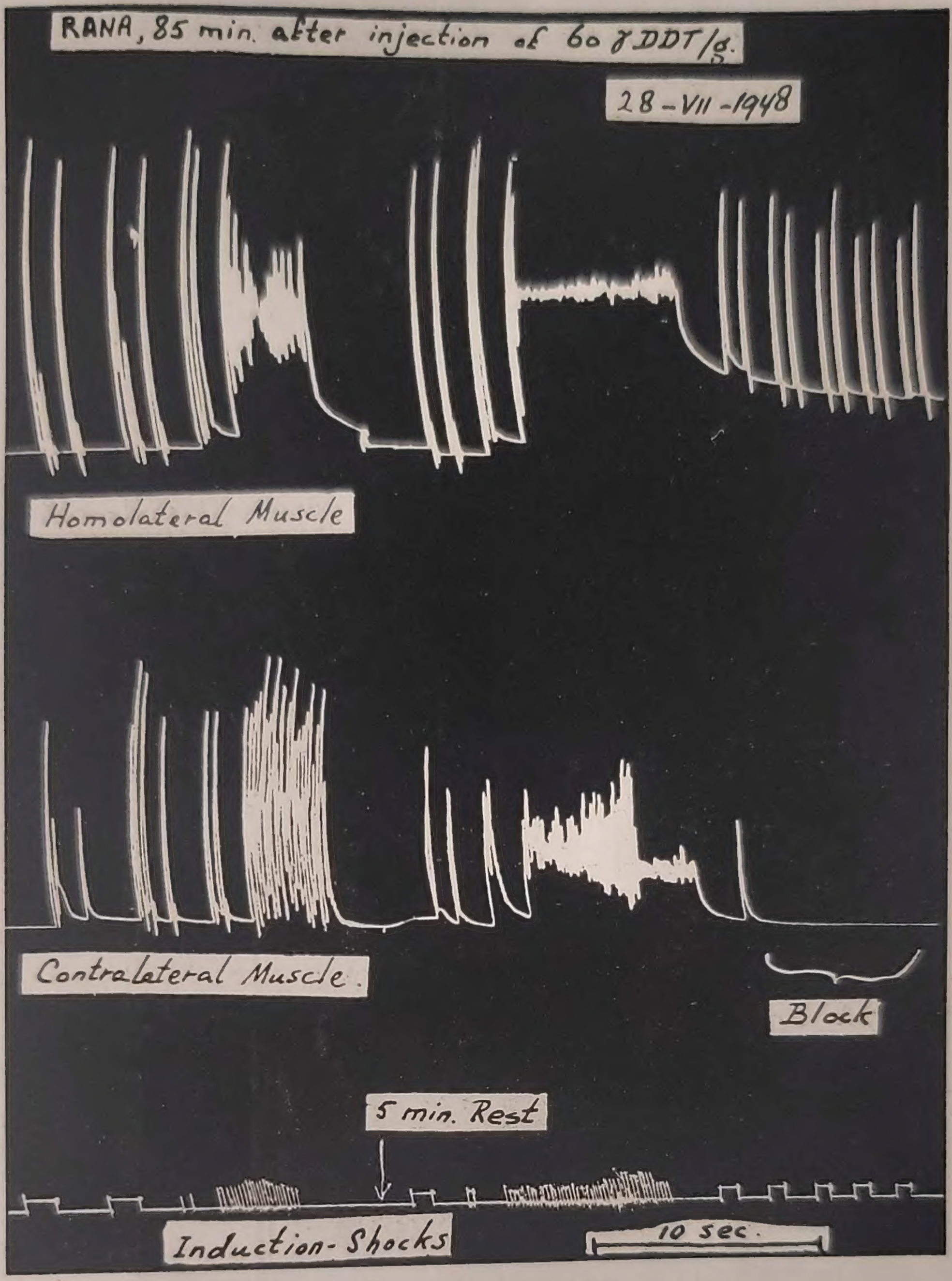


Fig. 28

Rana esculenta, mechanograms of the gastrocnemius muscles. For explanation see text p. 85 and 96.

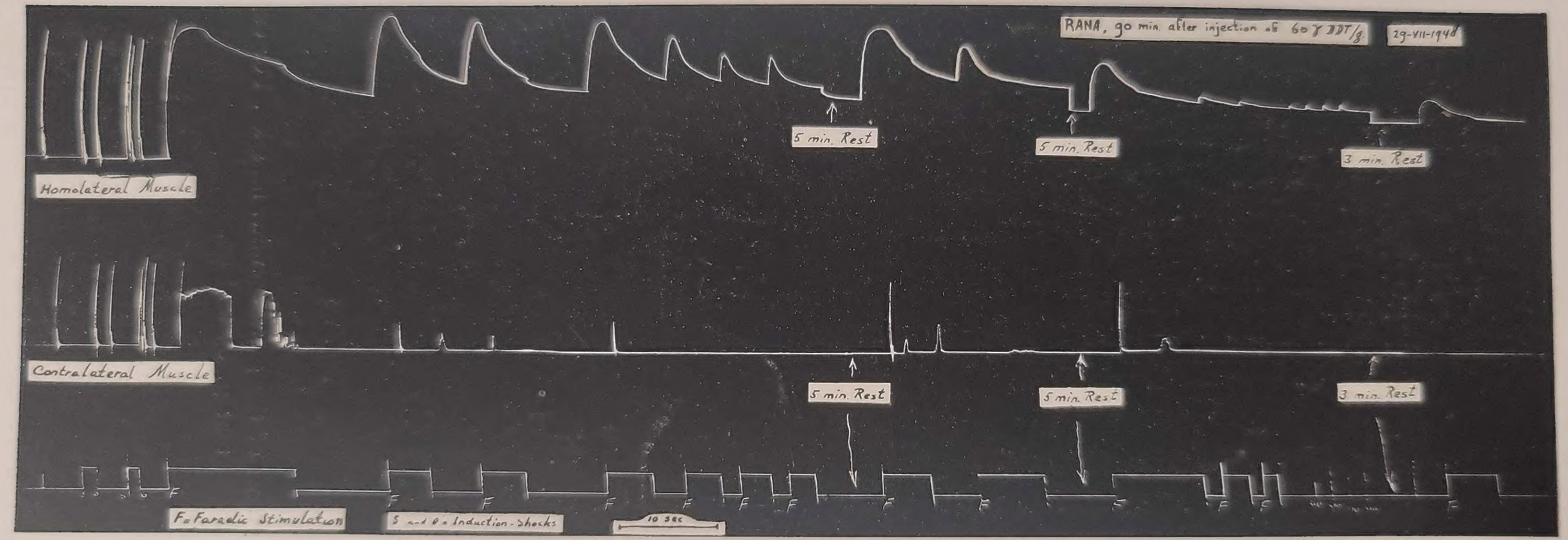


Fig. 29

Rana esculenta, mechanograms of the gastrocnemius muscles. For explanation see text p. 85 and 96.

Fig. 30

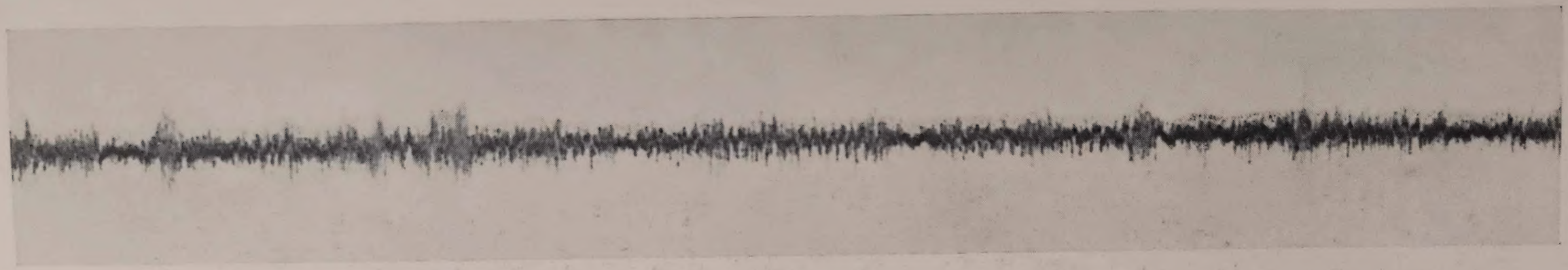


Fig. 31

Periplaneta americana, action potentials. Amplification the same as in Fig. 24. Speed of film see time signal I.
For explanation see text p. 98 (scale $^{3}/_{5}$).